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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C12N 15/12, C07K 14/705, 16/28, A61K 38/17

A2

(11) International Publication Number: WO 00/18912

(43) International Publication Date: 6 April 2000 (06.04.00)

(21) International Application Number: PCT/EP99/06991

(22) International Filing Date: 21 September 1999 (21.09.99)

(30) Priority Data:
60/101,706 25 September 1998 (25.09.98) US

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(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: ATP BINDING CASSETTE GENES AND PROTEINS FOR DIAGNOSIS AND TREATMENT OF LIPID DISORDERS AND INFLAMMATORY DISEASES

(57) Abstract

Modulation of the activity of transmembrane proteins belonging to the ATP binding cassette (ABC) transporter protein family which are etiologically involved in cholesterol driven atherogenic processes and inflammatory diseases like psoriasis, lupus erythematodes and others provides therapeutic means to treat such diseases. Furthermore, detection of herein identified ABC transporter proteins of their respective biochemical activities involved in such atherogenic and inflammatory processes provides diagnostic means for clinical application of diagnosis and monitoring of dyslipidemias, atherosclerosis or inflammatory diseases like psoriasis and lupus erythematodes.

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ATP binding cassette genes and proteins for diagnosis and treatment of lipid disorders and inflammatory diseases

Background of the invention

Reverse cholesterol transport mediated by HDL provides a "protective" mechanism for cell membrane integrity and foam cell formation and cellular cholesterol is taken up by circulating HDL or its precursor molecules. The precise mechanism of reverse cholesterol transport however is currently not fully understood and the mechanism of cellular cholesterol efflux and transfer from the cell surface to an acceptor-particle, such as HDL, is yet unclear. Certain candidate gene products have been postulated playing a role in the process of reverse cholesterol transport [1]. Apolipoproteins (e.g. ApoA-I, ApoA-IV), lipid transfer proteins (e.g. CETP, PLTP) and enzymes (e.g. LCAT, LPL, HL) are essential to exchange cholesterol and phospholipids in lipoprotein-lipoprotein and lipoprotein-cell interactions. Different plasma membrane receptors, such as SR-BI [2; 3], HB1/2 [4], and GPI-linked proteins (e.g. 120 kDa and 80 kDa) [5] as well as the sphingolipid rich microdomains (Caveolae, Rafts) of the plasma membrane have been implicated being involved in the process of reverse cholesterol transport and the exchange of phospholipids. How these membrane-microdomains are organized is in the current focus of interest for the identification of therapeutic targets. In recent studies SR-BI function as receptor for uptake of HDL into the liver and steroidogenic tissues could be demonstrated and the effectivity of this process is highly dependent on the phospholipid environment [2].

Cholesterol and phospholipid homeostasis in monocytes/macrophages and other cells involved in the atherosclerotic process is a critical determinant in atherosclerotic vessel disease. The phagocytic function of macrophages in host defense, tissue remodelling, uptake and lysosomal degradation of atherogenic lipoproteins and membrane fragments or other lipid containing particles has to be balanced by effective release mechanisms to avoid foam cell formation. HDL mediated reverse

cholesterol transport, supported by endogenous ApoE and CETP synthesis and secretion provides an effective mechanism to release excessive cholesterol from macrophages and other vascular cells.

Alternatively, reduced cholesterol and triglyceride/fatty acid absorption by intestinal mucosa cells as well as increased lipid secretion from hepatocytes into the bile will lower plasma lipids and the concentration of atherosclerotic lipoproteins.

Summary of the invention

New cholesterol responsive genes were identified with differential display method in human monocytes from peripheral blood that were subjected to macrophage differentiation and cholesterol loading with acetylated LDL and subsequent deloading with HDL_3.

In an initial screen ABCG1 (ABC8), a member of the rapidly growing family of ABC (ATP-Binding Cassette) transport systems, that couple the energy of ATP hydrolysis to the translocation of solutes across biological membranes, was identified as a cholesterol sensitive switch. ABCG1 is upregulated by M-CSF dependent phagocytic differentiation but expression is massively induced by cholesterol loading and almost completely set back to differentiation dependent levels by HDL_3.

In a more detailed analysis 37 already characterised ABC members and 8 Fragment - sequences (Table 2) were analysed in monocyte/macrophage cells by RT-PCR (linear range) for differentiation dependent changes and cholesterol sensitivity.

Among the 45 tested ABC-transporter genes 18 of the characterized ABC transporters and 2 of the Fragment -sequence based ABC-transporters are cholesterol sensitive (Example 4).

The cholesterol sensitive ABC-transporter are named according to the new ABC-

nomenclature and listed in Table 3 with the new and the old designations, respectively.

The most sensitive gene was ABCG1. ABCG1 is the human homologue of the drosophila white gene. Sequencing of the promoter of ABCG1 (Example 7) shows important transcription factor binding sites relevant for phagocytic differentiation and lipid sensitivity.

Antisense treatment of macrophages during cholesterol loading and HDL_3-mediated deloading clearly identified ABCG1 as a cholesterol transporter and the efflux of choline-containing phospholipids (phosphatidylcholine, sphingomyelin) was also modulated. Northern- and Western-blot analysis provided further support that inhibition of cholesterol transport is associated with lower ABCG1 mRNA expression and ABCG1 protein levels (Example 5).

Considerable evidence was derived from energy transfer experiments (Example 3) that ABCG1 in the cell membrane is in a regulated functional cooperation (e.g. cell differentiation, activation, cholesterol loading and deloading) with other membrane receptors that have either transport- (e.g. LRP-LDL receptor related protein) or signalling- and adhesion–function (e.g. integrins, integrin associated proteins) which is also supported by sequence homology of extracellular domains as well as other parts of the ABCG1 sequence. For example the protein sequence of the region of the third extracellular loop of ABCG1, i.e. aminoacid residues 580 through 644, shares homology with fibronectin (aa 317-327), integrinβ5 (aa 538-547), RAP (aa 119-127), LRP (aa 2874-2894), apoB-100 precursor (aa 4328-4369), glutathion-S-tranferase (aa 54-78) and glucose transporter (aa 371-380).Sequence comparison of all cholesterol sensitive transporters indicates this as a general principle of ABC transporter function and regulation.

Among the other cholesterol sensitive genes ABCA1 (ABC1) was further characterized. ABCA1 was identified in the mouse as an IL-1beta transporter

involved also in apoptotic cell processing. We show here, by RT-PCR (Table 2) and confirmation by Northern analysis, based on the newly detected human ABCA1 cDNA sequence (Example 6), that ABCA1 follows the same regulation as ABCG1.

Moreover, the ABCA1-knockout mice (ABCA1-/-) show massively reduced levels of serum lipids and lipoproteins. The expression of ABCA1 in mucosa cells of the small intestine and the altered lipoprotein metabolism in ABCA1-/- mice allows the conclusion that ABCA1 plays a major role in intestinal absorption and translocation of lipids into the lymph-system

Analysis of genetic defects that affect macrophage cholesterol homeostasis identified dysregulated ABCA1 as a gene locus involved in the HDL-deficiency syndrome (Tangier-Disease). This disease is associated with hypertriglyceridemia and splenomegaly.

Another as yet not described HDL-deficiency syndrome associated with early onset of coronary heart disease and psoriasis showed a dysregulation of the chromosome 17 associated ABC-sequences (ABCC4 (MRP3); ABCC3 (MRP3); ABCA5 (Fragment 90625); ABCA6 (Fragment 155051) :17q21-24). This points to an association with the predicted gene locus for psoriasis at chromosome 17.

A recently sequenced human ABC-transporter (ABCA8, Example 9) shows high homology to ABCA1 and also belongs to the group of cholesterol sensitive ABC-transporter.

ABCC5 (MRP5, sMRP) is a member of the MRP-subfamily among which ABCC2 (MRP2, cMOAT) was characterized as the hepatocyte canalicular membrane transporter that is involved in bilirubin glucoronide secretion [9] and identified as the gene locus for Dubin-Johnson Syndrome [10] a disorder associated with mild chronic conjugated hyperbilirubinemia.

Furthermore, the identification of ABCA1 as a transporter for IL-1 β identifies this gene as a candidate gene for treatment of inflammatory diseases including rheumatoid arthritis and septic shock. The cytokine IL-1 β is a broadly acting proinflammatory mediator that has been implicated in the pathogenesis of these diseases.

Moreover, we could demonstrate, that glyburide as an inhibitor of IL-1 β secretion inhibits not only Caspase I mediated processing of pro-IL-1 β and release of mature IL-1 β but simultaneously inhibits ceramide formation from sphingomyelin mediated by neutral sphingomyelinase and thereby releases human fibroblasts from G_2-phase cell cycle arrest. These data provide a further mechanism indicative for a function of ABCA1 in signalling and cellular lipid metabolism.

Autoimmune disorders that are associated with the antiphospholipid syndrome (e.g. lupus erythematodes) can be related to dysregulation of B-cell and T-cell function, aberrant antigen processing, or aberrations in the asymmetric distribution of membrane phospholipids. ABC-transporters are, besides their transport function, candidate genes for phospholipid translocases, floppases and scramblases that regulate phospholipid asymmetry (outer leaflet: PC+SPM; inner leaflet: PS+PE) of biological membranes [11]. There is considerable evidence for a dysregulation of the analysed ABC-transporters in patient cells. We conclude that these ABC-cassettes are also candidate genes for a genetic basis of antiphospholipid syndromes such as in Lupus erythematodes.

In summary, the ABC genes ABCG1, ABCA1 and the other cholesterol-sensitive ABC genes as specified herein, can be used for diagnostic and therapeutic applications as well as for biochemical or cell-based assays to screen for pharmacologically active compounds which can be used for treatment of lipid disorders, atherosclerosis or other inflammatory diseases. Thus it is an objective of the present invention to provide assays to screen for pharmacologically active compounds which can be used for treatment of lipid disorders, atherosclerosis or

other inflammatory diseases. Further the invention provides tools to identify modulators of these genes and gene products. These modulators can be used for the treatment of lipid disorders, atherosclerosis or other inflammatory diseases or for the the preparation of medicaments for treatment of lipid disorders, atherosclerosis or other inflammatory diseases. The medicaments comprise besides the modulator acceptable and usefull pharmaceutical carriers.

Abbreviations

aa	Amino acid
ABC	ATP-binding cassette
ABCA#	ATP-binding cassette, sub-family A (ABC1), member #
ABCB#	ATP-binding cassette, sub-family B (MDR/TAP), member #
ABCC#	ATP-binding cassette, sub-family C (CFTR/MRP), member #
ABCD#	ATP-binding cassette, sub-family D (ALD), member #
ABCE#	ATP-binding cassette, sub-family E (OABP), member #
ABCF#	ATP-binding cassette, sub-family F (GCN20), member #
ABCG#	ATP-binding cassette, sub-family G (WHITE), member #
ABCR	Homo sapiens rim ABC transporter
AcLDL	Acetylated LDL
ADP1	ATP-dependent permease
ALDP	Adrenoleukodystrophy protein
ALDR	Adrenoleukodystrophy related protein
ApoA	Apolipoprotein A
ApoE	Apolipoprotein E
ARA	Anthracycline resistance associated protein
AS	Antisense
ATP	Adenosine triphosphate
CETP	Cholesteryl ester transfer protein
CFTR	Cystic fibrosis transmembrane conductance regulator
CGT	ceramide glucoxyl transferase
CH	Cholesterol
cMOAT	Canalicular multispecific organic anion transporter
dsRNA	Double stranded RNA
Fragment	Gen Fragment
FABP	plasma membrane fatty acid binding protein

FACS	Fluorescence activated cell sorter
FATP	intracellular fatty acid binding protein
FCS	foetal calve serum
FFA	free fatty acids
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GCN20	protein kinase that phosphorylates the alpha-subunit of translation initiation factor 2
GPI	Glycosylphosphatidylinositol
HaCaT	keratinocytic cell line
HDL	High density lipoprotein
HL	Hepatic lipase
HlyB	haemolysin translocator protein B
HMT1	yeast heavy metal tolerance protein
HPTLC	High performance thin layer chromatography
IL	Interleukin
LCAT	Lecithin:cholesterol acyltransferase
LDL	Low density lipoprotein
LPL	Lipoprotein lipase
LRP	LDL receptor related protein
MDR	Multidrug resistance
MRP	Multidrug resistance-associated protein
PC	Phosphatidylcholine
PE	Phosphatidylethanolamin
PL	Phospholipid
PLTP	Phospholipid transferprotein
PMP	peroxisomal membrane protein
PS	Phosphatidylserine
RNA	Ribonucleic acid
RT-PCR	Reverse transcription – polymerase chain reaction
SDS	Sodium dodecyl sulfate

SL	Sphingolpid
sMRP	Small form of MRP
SPM	Sphingomyelin
SR-BI	Scavenger receptor BI
SUR	Sulfonylurea receptor
TAP	Antigen peptide transporter
TG	Triglycerides
TSAP	TNF-alpha stimulated ABC protein
UTR	untranslated region

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Description of the Figures

Figures 1 to 5 are showing nucleotide and protein sequences described in this application. The sequences are repeated in the sequence listing.

Description of Tabels:

Table 1:

Levels of RNA transcripts of ABCG1 (ABC8), ABCA1 (ABC1) and ABCA8 in human tissues were determined by Northern blot analysis of a multiple tissue dot-blot (Human RNA MasterBlot, Clontech Laboratories, Inc., CA, USA). The relative amount of expression is indicated by different numbers of filled circles.

Table 2:

The expression pattern of ABC-transporters in monocytes, monocyte derived macrophages (3 days cultivated monocytes in serum free Macrophage-SFM medium containing 50 ng/ml M-CSF), AcLDL incubated monocytes (3 days with 100 µg/ml) followed by HDL_3 (100 µg/ml) incubated monocytes is shown. Expressed genes are tested for cholesterol sensitivity by semiquantitative PCR.

For known ABC-Transporter the chromosomal location and the transported molecules are also presented.

Table 3:

Disorders, that are associated with ABC-transporters are shown. The chromosomal location is indicated and the relevant accession number in OMIN (Online Mendelian Inheritance in Man).

Table 4:

Expression of ABC-Transporters in HaCaT keratinocytic cells during differentiation

Table 1

Tissue	ABCG1 (ABC8)	ABCA1 (ABC1)
Adrenal gland	●●●●●	●●●
Thymus	●●●●	●●
Lung	●●●●	●●●
Heart	●●●	●●
Skeletal	●●	●
Brain	●●●	●●
Spleen	●●●●●	●●
Lymphnode	●●●	●
Pancreas	●	●
Placenta	●●●●	●●●●●
Colon	●●	●
Small intestine	●●	●●●●
Prostate	●●	●
Testis	●	●
Ovary	●●	●
Uterus	●	●●
Mammary gland	●●	●
Thyroid gland	●●	●●
Kidney	●●	●
Liver	●●●	●●●
Bone marrow	●	●
Peripheral leukocytes	●	●
Fetal tissue		
Fetal brain	●	●●
Fetal liver	●	●●●●
Fetal spleen	●●	●●●
Fetal thymus	●●	●●
Fetal lung	●●	●●●

Table 2: Cholesterol dependent gene regulation of human ABC transporters

Gene	chromosomal localization	peripheral blood monocytes	3 days old M-CSF M□	cholesterol loading (acLDL)	cholesterol deloading (HDL3)	transported molecules
ABCG1 (ABC8)	21q22.3	+	↑	↑↑	↓↓	cholesterol / choline PL
ABCA1 (ABC1)	9q22-31	+	↑	↑↑	↓↓	cholesterol / IL-1□
ABCC5 (MRP5)	3q25-27	+	↑	↑↑	↓	
ABCD1 (ALDP, ALD)	Xq28	+	↑	↑	↓	very long chain fatty acids
ABCA5 (est90625)	17q21-25	+	↑	↑	↓	
ABCB11 (BSEP, SPGP)	2q24	+	↑	↑↑	↓	bile acids
ABCA8 (ABC-new)		+	+	↑	↓	
ABCC2 (MRP2)	10q23-24	+	+	↑	↓	bilirubin glucuronide
ABCB6 (est45597)	2q33-36	+	+	↑	↓	
ABCC1 (MRP1)	16p13.12	+	↓	↑	↓	eicosanoids
ABCA3 (ABC3)	16p13.3	+	↑	↑	nr	
est1133530		+	↑	↑	nr	
ABCB4 (MDR3)	7q21	+	↑	↓	↑	phosphatidylcholine
ABCG2 (est157481,ABCP)	4q22-23	+	↑	↓	↑	
ABCC4 (MRP4)	13q31	+	↑	↓	↑	
ABCB9 (est122234)	12q24	+	↑	↓	↑	
ABCD2 (ALDR)	12q11	+	↓	↓	↑	very long chain fatty acids
ABCB1 (MDR1)	7q21	+	+	↓	↑	phospholipids,amphiphiles
ABCA6 (est155051)	17q21	+	↑	↓	nr	
est640918		+	↑	↓	nr	
ABCD4 (P70R)	14q24.3	+	↑	nr	nr	
ABCA2 (ABC2)	9q34	+	↑	nr	nr	
ABCF2 (est133090)	7q35-36	+	↑	nr	nr	
ABCB7 (ABC7)	Xq13.1-3	+	↑	nr	nr	iron
ABCF1 (ABC50,TSAP)	6p21.33	+	↑	nr	nr	
ABCC6 (MRP6)	16p13.11	+	↓	nr	nr	
ABCB5 (est422562)	7p14	+	↓	nr	nr	
ABCC3 (MRP3)	17q11-21	+	nr	nr	nr	
ABCA4 (ABCR)	1p22	+	nr	nr	nr	retinoids, lipofuscin
ABCB2 (TAP1)	6p21.3	+	nr	nr	nr	peptides
ABCB3 (TAP2)	6p21.3	+	nr	nr	nr	peptides

Gene		chromosomal localization	peripheral blood monocytes	3 days old M-CSF M□	cholesterol loading (acLDL)	cholesterol deloading (HDL3)	transported molecules
ABCF3	(est201864)	3q25.1-2	+	nr	nr	nr	
ABCB8	(est328128)	7q35-36	+	↑	nr	nr	
ABCE1	(OABP)	4q31	+	↑	nr	nr	
ABCB10	(est20237)	1q32	+	↑	nr	nr	
est698739			+	↑	nr	nr	
ABCC10	(est182763)	6p21	+	nr	nr	nr	
ABCC7	(CFTR)	7q31	∅	∅	∅	∅	ions
ABCC8	(SUR-1)	11p15.1	∅	∅	∅	∅	
ABCD3	(PMP70)	1p21-22	∅	∅	∅	∅	
Huwhite2			∅	∅	∅	∅	
est1125168			∅	∅	∅	∅	
est1203215			∅	∅	∅	∅	
est168043			∅	∅	∅	∅	
est990006			∅	∅	∅	∅	

+ = expressed ∅ = not expressed

nr=not regulated ⇑ = upregulated ⇓= downregulated

half (hs) or full size (fs) transporter as deduced from the mRNA size

Table 3

Disorders	*Genomic location*	*Associated gene*	OMIM-acc.nr.
Metabolic disorders:			
Cystic fibrosis	7q31.3	ABCC7 (CFTR)	219700
Dubin Johnson syndrome (mild chronic conjugated hyperbilirubinemia)	10q24	ABCC2 (CMOAT)	237500
Progressive familial intrahepatic cholestasis type III (PIFC3)	7q21.1	ABCB4 (MDR3)	602347
Byler disease (PFIC2)	***2q24***	***ABCB11 (BSEP, sPGP)***	***601847***
Familial persistent hyperinsulinemic hypoglycemia	11p15.1	ABCC8 (SUR-1)	601820
IDDM	6p21.3	ABCB2 (TAP1)/ABCB3 (TAP2)	222100
Neuronal disorders:			
Adrenoleukodystrophy	12q11	ABCD2 (ALDR)	300100
Zellweger's syndrome	1p22-21	ABCD3 (PMP70)	214100
Multiple Sclerosis	6p21.3	ABCB2 (TAP1)/ABCB3 (TAP2)	126200
X-linked Sideroblastic anemia with spinocerebellar ataxia	Xq13.1-3	ABCB7 (ABC7)	301310
Menkes disease (altered homeostasis of metals)	Xq13	ABCB7 (ABC7)	309400
Immune/Hemostats disorders:			
Herpes simplex virus infection [12]	6p21.3	ABCB2 (TAP1)/ABCB3 (TAP2)	
Behcet's syndrome	6p21.3	ABCB2 (TAP1)/ABCB3 (TAP2)	109650
Bare lymphocyte syndrome type I	6p21.3	ABCB2 (TAP1)/ABCB3 (TAP2)	209920
Scott syndrome	7q21.1	ABCB1 (MDR1)	262890
Retinal dystrophies:			
Fundus flavi maculatus with macular dystrophy	1p13-21	ABCA4 (ABCR)	601691
Juvenile Stargardt disease	1p13-21	ABCA4 (ABCR)	248200
Age-related macular degeneration	1p13-21	ABCA4 (ABCR)	153800
Cone-rod dystrophy	1p13-21	ABCA4 (ABCR)	600110
Retinitis pigmentosa	1p13-21	ABCA4 (ABCR)	601718

Diseases with evidence for involvement of ATPcassettes/translocases and floppases[80]		*Assumed gene*	
BRIC (Benign recurrent intrahepatic obstructive jaundice)	18	Assumed	243300
Psoriasis	17q11-12 17q21-24	ABCA5 (Fragment 90625) ABCC3 (MRP3)	602723 177900 601454
Lupus erythematodes – Antiphospholipid Syndrome		Translocase Flippase	152700
PFIC(Prog. Fatal familial intrahepatic choestasis) PFIC1	18q21-22	ATP Transporters	211600
Neurological disorders mapped to gene locus of ABCG1 (ABC8)			
Autosomal bipolar affective disorder	21q22.3	ABCG1 (ABC8)	125480
Autosomal recessive non-syndromic deafness	21q22.3	ABCG1 (ABC8)	601072
Down Syndrome (ABC-8 may be a candidate for the Brushfield spots – mottled, marble or speckled irides frequently seen in Down-Syndrome) Linkage to phosphofructokinase (liver type)	21q22.3 21q22	ABCG1 (ABC8)	190685 171860
HDL-deficiency syndromes, Gen responsible for Tangier Disease	9q31	ABCA1 (ABC1)	205400

Table 4: Expression of ABC-Transporters in HaCaT keratinocytic cells during differentiation

Gene	**chrom. localisation**	**initial expression**	**differentiation dependent expression**	**known or putative molecules transported**
ABCG1 (ABC8)	21 q22.3	+++++	↑	cholesterol choline-PL
ABCC3 (MRP3)	17 q11-q12	+++++	↑	
ABCA8	19 P13	+++++	↑	
ABCC1 (MRP1)	16 p13	+++++	↗ ↘ (max. day 2)	PGA_2, LTC_4 DNP-SG
ABCD4 (PMP69, P70R)	14 q24	+++++	↗ ↘ (max. day 2,4)	
ABCC2 (MRP2)	10 q24	+++	↗ ↘ (max. day 2)	bilirubin glucuronide
ABCA3 (ABC3)	16 p13	+	↗ ↘ (max. day 4,6)	
ABCA5 (ABCR)	1 p21	+	↗ ↘ (max. day 4)	retinoid, lipofuscin
ABCA1 (ABC1)	9 q22-q31	+	↗ ↘ (max. day 6)	
ABCC6 (MRP6)	16 p13.11	+	↗ ↘ (max. day 4)	
ABCC4 (MRP4)	13 q31	++++	↗ ↘ (max. day 2,4)	
ABCA2	9 q34	++++	↗ ↘ (max. day 6)	
ABCC5 (MRP5, SMRP)	3 q27	+++++	↗ ↘ (max. day 2,4)	

ABCB6 (est45597)	2	+++++	↗ ↘ (max. day 2,4)	
ABCB7 (ABC7)	X q13.1-3	+++++	↗ ↘ (max. day 4)	irons
TAP1 (ABCB1)	6 p21.3	+++++	↗ ↘ (max. day 4,6)	peptides
TAP2 (ABCB2)	6 p21.3	+++++	↗ ↘ (max. day 2,4)	peptides
ABCB8 (est328128)	7 q35-36	+++++	↗ ↘ (max day 2)	
EST640918	17 q24	+	↗ ↘ (max day 4)	
ABCC7 (CFTR)	7 q31	+++	↗ ↘ (max day 4)	
ABCB10 (est20237)	1 q32	+++	↗ ↘ (max. day 2)	
ABCF1 (TSAP)	6 p21.33	+++++	↓	
ABCC10 (est182763)	1 q32	+++++	↓	
ABCE1 (OABP)	4 q31	+++++	↓	
EST698739	17 q24	+++++	↓	
ABCF2 (est133090)	7 q35-q36	+++++	↓	
ALD (ABCD1,ALDP)	X q28	++++	↓	VLCFA
ABCA5 (est90625)	17 q21-q24	+++	↓	
ABCB5 (est422562)	7 p14	++++	↓	
ABCB9 (est122234)	12 q24-q_{ter}	++	↓	
ABCD2 (ALDR)	12 q11	+	↓	VLCFA
ABCF3 (est201864)	3 q25.1-2	+++++	↓	
ABCG2 (ABC15,ABCP)	4 q22-q23	++++	↓	
EST1133530	4 p16pter	+++++	↓	

Huwhite	11 q23	+++++	↓	
ABCA6 (est155051)	17 q21	++	↓	
BSEP (ABCB11,sPGP)	2 q24	+	↓↑ (max day 6)	
ABCB4 (MDR3)	7 q21	not expressed		phosphatidyl-choline
ABCD3 (PMP70)	1 p22	not expressed		
ABCB1 (MDR1)	7 q21	not expressed		phospholipids amphiphiles
EST168043	2 p15-16	not expressed		
EST990006	17 q24	not expressed		
ABCC8(SUR1)	11 p15.1	not expressed		

+: relative expression n.d.: not determined

↑: upregulated ↓: downregulated ↗ ↘: biphasic expression

Description of specific embodiments

Candidate gene identification during cholesterol loading and deloading of human monocyte derived macrophages

In order to discover genes that are involved in the cholesterol loading and/or deloading in vitro assays were set up. Particularly, gene expression in human blood derived monocytes and macrophages elicited by cholesterol and its physiological transport formulation, i.e. various low density lipoprotein (LDL) particle species like AcLDL, was studied.

Elutriated human monocytes were cultivated in M-CSF containing but serum free macrophage medium supplemented with AcLDL (100 μg protein/ml medium) for three days, followed by cholesterol depletion replacing AcLDL by HDL_3 (100 μg protein/ml medium) for twelve hours. Differential display screening for new candidate genes, regulated by cholesterol loading/deloading, was performed (Example 1).

Identification of a new cholesterol sensitive gene

ABCG1 (ABC8) was discoverd as a novel cholesterol sensitive gene. ABCG1 belongs to the ATP binding cassette (ABC) transporter gene family. ABCG1 was recently published as the human analogue of the drosophila white gene [6-8].

The gene is strongly upregulated by AcLDL-mediated cholesterol loading, and almost completely downregulated by HDL_3 mediated-cholesterol deloading, as confirmed by Northern blot (Example 2). Nothern blot analysis oh mRNA from human monocyte-derived macrophages obtained from the peripherical blood probands clearly show upregulation of ABCG1 mRNA formation upon AcLDL incubation. In sharp contrast, ABCG1 mRNA expression was decreased in such macrophages upon incubation with HDL_3 containing medium.

ABCG1 expression in cholesterol loaded and deloaded cells after four days pre-differentiation

For effective cholesterol loading monocytes must be differentiated to phagocytic-macrophage like cells. During this period scavenger receptors are upregulated and promote AcLDL uptake leading to cholesteryl ester accumulation. After four days preincubation period we have incubated the cells for one, two and three days with AcLDL (100 µg/ml) to show cholesteryl ester accumulation. After two days of loading we deloaded the cells with HDL_3 for 12 hours, 24 hours and 48 hours, respectively. ABCG1 is time dependently upregulated during the AcLDL loading period and downregulated by HDL_3 deloading (Examples 2 and 3) In order to confirm time dependent increase of ABCG1 mRNA expression after AcLDL challenge in human monocyte derived macrophages, Nothern blot analyses for ABCG1 mRNA quantification were made, RNA samples from the macrophages were harvested at day zero and day four as controls and mRNA samples were taken one, two, and three days after AcLDL treatment of macrophages, which started at day four. A dramatic increase of ABCG1 mRNA content of the macrophages could be detected from day five through day seven by Nothern blot analyses.

This regulation shows the same pattern as changes of cellular cholesteryl ester content (Example3). Cholesterol ester accumulation starts in monocyte-derived macrophages upon AcLDL stimulation from a base level below 5 nmol/mg cell protein at day four up to 120 nmol/mg cell protein at day seven (i.e. three days after AcLDL application).

Tissue expression

Besides cholesterol loaded macrophages ABCG1 is prominently expressed in brain, spleen, lung, placenta, adrenal gland, thymus and fetal tissues (Table 1).

Chromosomal location and associated genes and diseases

The ABCG1 gene maps to human chromosome 21q 22.3. Also localized in this region 21q 22.3 are the following genes: integrin β 2 (CD18), brain specific polypeptide 19, down syndrome cell adhesion molecule, dsRNA specific adenosine deaminase, cystathionine β synthase, collagen VI alpha-2, collagen XVIII alpha-1, autosomal recessive deafness, and amyloid beta precursor.

This chromosomal region is in close proximity to other regions involved in Down syndrome, autosomal dominant bipolar affective disorder, and autosomal recessive non-syndromic deafness.

Extracellular loop of ABCG1 (ABC8) for antibody generation

The putative structure of the hydrophobic transmembrane region of ABCG1 shows 6 transmembrane spanning domains, and 3 extracellular loops, two of them are 9- and 8-amino acids-long, respectively, while the third one is 66-amino acids-long.

The larger one of the two intracellular loops consists of 30 amino acids. Similarity-survey in protein databases for homologies the 3rd extracellular loop (IIIex) with other genes resulted in the identification of fibronectin, integrinβ5, RAP, LRP (LDL receptor related protein) apo-lipoprotein B 100 precursor protein, glutathion S-transferase and glucose transporter.

A polyclonal antiserum was generated against the 3rd extracellular loop (IIIex) of ABCG1 in order to perform flow cytometric analysis, energy transfer experiments and Western-blotting (see Example 3). In the amino acid sequence of ABCG1 the 3rd extracellular loop (IIIex) comprises 66 amino acids comprises 66 amino acids from amino acid 580 through 644. The peptide fragment for antibody generation comprises the amino acid residues 613 through 628 of ABCG1 polypeptide. ABCG1 obviously interacts with endogenous sequence motivs with other membrane receptors

involved in transport (e.g. LRP, RAP), signalling and adhesion (e.g. integrins, integrin associated proteins) as a basis of ABCG1-function and regulation. Moreover sequence comparisons of all ABC-transporters listed in Table 3 indicates functional cooperation with other membrane receptors as a general principle of the whole gene family.

Subfamily-Analysis

Evolutionary relationship studies with the whole ABC transporter family have shown that ABCG1 (ABC8) forms a subfamily together ABCG2 (est157481) and this subfamily is closely related to the full-size transporters ABCA1 (ABC1), ABCA2 (ABC2), ABCA3 (ABC3), ABCA4 (ABCR) and the half-size transporter ABCF1 (TSAP) .

Recent studies by Allikmets et al. have identified 21 new genes as ABC transporters by expressed sequence tags database search [13].

General description of the ABC transporter family

The ATP-binding cassette (ABC) transporter superfamily contains some of the most functionally diverse proteins known. Most of the members of the ABC family (also called traffic ATP-ases) function as ATP-dependent active transporters (Table 3). The typical functional unit consists of a pair of ATP-binding domains and a set of transmembrane (TM) domains. The TM-domains determine the specificity for the type of molecule transported, and the ATP-binding domains provide the energy to move the molecule through the membrane [14; 15]. The variety of substrates handled by different ABC-transporters is enormous and ranges from ions to peptides. Specific transporters are found for nutrients, endogenous toxins, xenobiotics, peptides, aminoacids, sugars, organic/inorganic ions, vitamins, steroid hormones and drugs [16; 17].

ABC-transporter associated diseases

The search for human disease genes (Table 3) provided a number of previously undiscovered ABC proteins [16]. The best characterized disease caused by a mutation in an ABC transporter is cystic fibrosis (ABCC7 (CFTR)). Inherited disorders of peroxisomal metabolism as Adrenoleukodystrophy and Zellweger's syndrome also show alterations in ABC transporters. They are involved in peroxisomal beta-oxidation, necessary for very long chain fatty acid metabolism [18].

Antisense against ABCG1 inhibits cholesterol efflux to HDL_3

Since ABCG1 is a cholesterol sensitive gene and other ABC transporters are known to be involved in certain lipid transport processes, the question arises whether ABCG1 plays a role in transport of cholesterol, phospholipids, fatty acids or glycerols. Therefore antisense experiments were performed to test the influence of ABCG1 on lipid loading and deloading. The inhibition of ABCG1 with specific antisense oligonucleotides decreased the efflux of cholesterol and phosphatidylcholine to HDL_3. (Example **5)**

Other cholesterol sensitive ABC transporter

Cloning and sequencing of the human ABCA1 (ABC1) provided the information to characterize ABCA1 for cholesterol sensitivity, and tissue distribution (Example 6). Another cholesterol sensitive human ABC transporter (ABCA8) has been cloned and sequenced (Example 8)

Characterization of the ABCG1 promoter region

The ABCG1 promoter has the characteristic binding sites for transcription factors that are involved in the differentiation of monocytes into phagocytic macrophages. The cholesterol sensitivity of the expression of ABCG1 is represented by the transcription factor pattern that is relevant for phagocytic differentiation (Example 7).

Examples

Example 1

Identification of cholesterol loading and deloading candidate genes

Monocyte isolation and cell culture

Monocytes were obtained from peripheral blood of healthy normolipidemic volunteers by leukapheresis and purified by counterflow elutriation. Purity of isolated monocytes was >95% as revealed by FACS analysis. $10x10^6$ monocytes were seeded into 100 mm^2 diameters cell culture dishes under serum free conditions in macrophage medium for 12 hours in a humidified 37°C incubator maintained with a 5% CO2, 95% air atmosphere. After 12 hours medium containing unattached cells was replaced by fresh macrophage medium supplemented with 50 ng/ml human recombinant M-CSF (this medium is the standard medium for any further incubations).

Isolation of lipoproteins and preparation of AcLDL

Lipoproteins were prepared from human plasma from healthy volunteer donors by standard sequential ultracentrifugation methods in a Beckman L-70 ultracentrifuge equipped with a 70 Ti rotor at 4°C to obtain LDL (d=1,006 to 1,063 g/ml) and HDL_3 (d=1,125 to 1,21 g/ml). All densities were adjusted with solid KBr. Lipoprotein fractions are extensively dialyzed with phosphate-buffered saline (PBS) containing 5 mM EDTA. The final dialysis step was in 0,15 mol/L NaCl in the absence of EDTA. Lipoproteins were made sterile by filtration through a 0.45 μm (pore-size) sterile filter (Sartorius).

LDL was acetylated by repeated addition of acetic anhydride followed by dialysis against PBS [19]. Modified LDL showed enhanced mobility on agarose gel electrophoresis.

Incubation of monocyte-macrophages with AcLDL and HDL_3

After 12 hours of preincubation cells were grown in the presence or absence (control) of 100 μg protein /ml AcLDL for further 3 day in medium. Then, the incubation medium was replaced with fresh medium and incubated with or without the addition of HDL_3 (100 μg/ml) for another 12 hours.

Differential display

Differential display screening was performed for new candidate genes that are regulated by cholesterol loading/deloading as described [20; 21]. In brief, 0,2 μg of total RNA isolated from monocytes at various incubations was reverse transcribed with specific anchored oligo-dT primers, using a commercially available kit (GeneAmp RNA PCR Core Kit, Perkin Elmer, Germany). The oligo-dT primers used had two additional nucleotides at their 3' end consisting of an invariable A at the second last position (3'-end) and A, C, G or T at the last position to allow a subset of mRNAs to be reverse transcribed. Here, a 13-mer oligo-dT (T101: 5'T11AG-2') was used in a 20-μl reaction at 2,5 μM concentration. One tenth of the cDNA was amplified in a 20-μl PCR reaction using the same oligo-dT and an arbitrary 10-mer upstream primer (D20 5'-GATCAATCGC-3'), 2,5 μM each, using 2,5 units of TAQ DNA Polymerase and 1.25 mM MgCl2. Amplification was for 40 cycles with denaturation at 94°C for 30 sec, annealing at 41°C for 1 min and elongation at 72°C for 30 sec with a 5 min extension at 72°C following the last cycle. All PCR reactions were carried out in a Perkin Elmer 9600 thermocycler (Perkin Elmer, Germany). PCR-products were separated on ready to use 10% polyacrylamide gels with a 5% stacking gel (CleanGel Large-10/40 ETC, Germany) under non-denaturating conditions using the Multiphor II electrophoresis apparatus (Pharmacia, Germany). The DNA fragments were visualized by silverstaining of the gel as previously described [22].

Cloning and sequencing of differentially expressed cDNAs

cDNA bands of interest were cut out of the gel and DNA was isolated by boiling the gel slice for 10 min in 20 µl of water. A 4 µl aliquot was used for the following PCR-reaction in a 20µl volume. The cDNA was reamplified using the same primer set and PCR conditions as above, except, that the final dNTP concentration was 1mM each. Reamplified cDNAs were cloned in the pUC18-vector using ABCC8 (SUR)eClone-Kit (Pharmacia), sequenced on an automated fluorescence DNA sequencer using the AutoRead Sequencing Kit (Pharmacia, Germany) and used as probes for Northern blot analysis [23].

Example 2

Northern Blot analyses of monocytes and macrophages after 3 days AcLDL incubation followed by 12 hours HDL_3 incubation

Elutriated monocytes were incubated with AcLDL (100 µg/ml medium) for 2.5 days or differentiated for the same time without the addition of AcLDL as control. ABCG1 (ABC8) expression is 4 times stronger upregulated with AcLDL incubation than in differentiated monocytes .After the AcLDL incubation period cells were washed and incubated with HDL_3 for the next 12 hours or with medium alone as control. ABCG1 expression is almost completely downregulated by HDL3 incubation and only moderatly decreased in control incubation as confirmed by Northern blot. For effective cholesterol loading monocytes must be differentiated to macrophage like cells. During this period scavenger receptors are upregulated and promote AcLDL uptake leading to cholesteryl ester accumulation. To differentiated the cells prior to AcLDL-dependent cholesterol loading, we cultured the cells for four days in standard medium. At day four, cells were washed and incubated with AcLDL (100µg/ml medium) or in the absence of AcLDL as control for further one, two and three days to load the cells with cholesterol. At each timepoint cells were lysed with 0.1 % SDS and lipid was extracted as described in materials and methods and cellular cholesteryl ester was determined by HPTLC-separation. Cells were loaded time

dependently up to 120 nmol/mg cell protein after 3 days AcLDL loading, whereas in unloaded cells no cholesteryl ester accumulation could be observed.

To distinguish HDL_3 dependent and independent cholesterol efflux cells were pulsed with AcLDL (100 µg/ml) for three days with the coincubation of ^{14}C-cholesterol (1,5 µCi/ml medium). Cells were washed and deloaded with HDL_3 (100 µg/ml) for 12 hours, 24 hours and 48 hours, respectively. Cells were incubated without the addition of exogenous lipid-acceptors as a control. After chase period the content of ^{14}C-cholesterol was determined in the medium and in the cells by liquid scintillation as described in material and methods. The efflux of cholesterol is expressed in percent of cellular DPMs of total DPMs (counts in the cells plus medium) With HDL_3 the efflux is faster and more intense. than the efflux without the addition of HDL_3 as an endogenous lipid acceptor. After 12 hours cellular cholesterol content was reduced to 68 % with HDL_3-dependent deloading, and 86 % in HDL_3-independent deloading. After 48 hours only 35 % of loaded 14C-cholesterol was observed in the cells treated with HDL_3 . In contrast, 70 % of loaded ^{14}C-cholesterol was found in untreated cells

In AcLDL pulsed cells the RNA-expression of ABCG1 is upregulated whereas no upregulation appears in the cells that were not loaded with AcLDL. Cells that were loaded for two days with AcLDL were deloaded with HDL_3 for 12. 24 and 48 hours (12h; 24h; 48h), and in the absence of exogenous lipid acceptors. The RNA-expression is downregulated again, in HDL_3 treated cells more intense than in cells treatet without any exogenous lipid acceptor.

Materials:

Macrophage medium (Macrophage-SFM) was obtained from Gibco Life Technologies, Germany. Human recombinant M-CSF was obtained from Genzyme Diagnostics, Germany, and antisense phosphorothioate oligonucleotides were supplied by Biognostics, Germany. All other chemicals were purchased from Sigma. Nylon membranes and a32P-dCTP were obtained from Amersham, Germany, 14C-

cholesterol and 3H-choline chloride from NEN, Germany, and cell culture dishes are Becton Dickinson, Germany

Isolation of total RNA and northern blotting

Total RNA was isolated at each time-point, before and after AcLDL incubation, and after HDL_3 incubation, respectivly, Washed cells were solubilized in guanidine isothiocyanate followed by sedimentation of the extract through cesium chloride [24]. For Northern analysis, 10 µg/lane of total RNA samples were fractionated by electrophoresis in 1,2% agarose agarose gel containing 6% formaldehyde and blotted onto nylon membranes (Schleicher & Schüll, Germany). After crosslinking with UV-irradiation (Stratalinker model 1800, Stratagene, USA), the membranes were hybridized with a cDNA probe for ABCG1 (ABC8). Hybridization and washing conditions were performed as recommended by the manufacturer of the membrane.

Example 3

Westernblot analysis of monocytes and macrophages after cholesterol loading and deloading

Protein expression of ABCG1 (ABC8) is upregulated in AcLDL-loaded and down-regulated in HDL_3-deloaded monocyte-derived macrophages. Western blotting with a peptide antibody against ABCG1 as described in materials and methods is performed with 40 µg of total protein for each lane of SDS-PAGE. ABCG1-protein expression is shown in freshly isolated monocytes (day zero) and in differentiated monocytes (day four). From day four to day seven (5d; 6d; 7d) monocyte-derived macrophages were loaded with AcLDL or without AcLDL as control. AcLDL loaded cells from day 6 (6d) were deloaded with HDL_3 for 12, 24, and 48 hours and without exogenous added HDL lipid-acceptor. AcLDL increases the protein-expression, whereas HDL_3 decreases the expression to normal levels again.

Protein isolation and determination

At each timepoint cells were lysed with 0.1% SDS and the protein content was determined by the method of Lowry et al. [25].

Generation of ABCG1 specific antibodies

ABCG1 specific peptide antibodies were generated by immunization of chickens and rabbits with a synthetic peptide (Fa. Pineda, Berlin). The peptide sequence was chosen from the extracellular domain exIII amino acid residues 613-628 of ABCG1 comprising the amino acids REDLHCDIDETCHFQ (see sequence listing ID No. 53). After 58 days of immunization western blotting was performed with 1:1000 diluted serum and 1:10000 secondary peroxidase labelled antibody.

Electrophoresis and immunoblotting

SDS-polyacrylamide gelelectrophoresis was performed with 40µg total cellular protein per lane. Proteins were transferred to Immobilon as reported. Transfer was confirmed by Coomassie Blue staining of the gel after the electroblot. After blocking for at least 2 hours in 5% nonfat dry milk the blot was washed 3 times for 15 minutes in PBS. Antiserum generated as described was used at 1:1000 dilution in 5% nonfat dry milk in PBS. The blot was incubated for 1 hour. After 4 times washing with PBS at room-temperature a secondary peroxidase-labelled rabbit anti chicken IgG-antibody (1:10000 diluted, Sigma) was incubated in 5% nonfat dry milk in PBS for 1 hour. After 2 times washing with PBS, detection of the immune complexes was carried out with the ECL Western blot detection system (Amersham International PLC, UK).

Fluorescence resonance energy transfer:

Monocytes were labelled with the specific antibodies for 15 minutes on ice, one antibody is labelled by biotin, the other one is labelled by phycoerythrin. After washing the cells were incubated with a Cy5-conjugated streptavidin for another 15 minutes.

Distances between antibody labelled proteins on the cell surface is measured by energy transfer with a FACScan (Becton Dickinson). Following single laser excitation at 488 nm the Cy5 specific emmission represents an indirect excitation of Cy5 dependent on the proximity of the PE-conjugated antibody. The relative transfer efficiency was calculated following standardisation for the intensity of PE and Cy5 labelling and nonspecific overlap of fluorescence based on dual laser excitation and comparison to separately stained control samples.

Example 4

Cholesterol sensitivity of ABCG1 (ABC8) and other members of the ABC-transporter family

The influence of cholesterol loading and deloading on other members of the ABC-family was also investigated to find out the potential second half-size ABC transporter.

Further analysis has been performed to examine the expression pattern of all human ABC transporters in monocytes and monocyte derived macrophages as well as in cholesterol loaden and deloaden mononuclear phagocytes.

The experiments were performed by RT-PCR with cycle-variation to compare the expression in the quantitative part of the distinct PCR. Primer sets were generated from the published sequences of the ABC-transporters. A RT-PCR with GAPDH primers was used as control.

Several ABC-transporters are also cholesterol sensitive which further supports the function of ABC-transporters in cellular lipid trafficking (Table 2).

Semi-quantitative RT-PCR

All known ABC-transporters are tested for AcLDL/HDL_3 sensitive regulation of expression using RT-PCR with cycle-variation to compare the expression in the

quantitative part of the distinct PCR. 1 µg of total RNA was used in a 40 µl reverse transcription reaction, using the Reverse Transkription System (Promega, Corp. WI, USA). Aliquots of 5 µl of this RT-reaction was used in 50µl PCR reaction. After denaturing for 1,5 min at 94°C, 35 or less cycles of PCR were performed with 92,3°C for 44s, 60,8°C for 40s (standard annealing temperature differs in certain primer-combinations), 71,5°C for 46s followed by a final 5-min extension at 72°C. The Primer sets were generated from the published sequences of the ABC-transporters. A RT-PCR with primers specific for GAPDH was performed as control.

The expression pattern of ABC-transporters in monocytes, monocyte derived macrophages (3 days cultivated monocytes in serum free macrophage-SFM medium containing 50 ng/ml M-CSF), AcLDL incubated monocytes (3 days with 100 µg/ml) followed by HDL_3 (100 µg/ml) incubated monocytes is shown in Table 2. Expressed genes are tested for cholesterol sensitivity by semi-quantitative PCR.

Example 5:

Functional analyses of the cholesterol sensitive ABCG1 (ABC8) transporter gene by antisense oligonucleotide experiments

Antisense experiments were conducted in order to address the question. that beyond being regulated by cholesterol loading and deloading ABCG1 is directly involved in lipid loading and deloading processes.

In various experiments antisense oligonucleotides decreased the efflux of cholesterol and phosphatidylcholine to HDL_3. During the loading period with AcLDL the cells were coincubated with *17* different antisense oligonucleotides. To measure the efflux of cholesterol and phospholipids the cells were pulsed in the loading period with 1,5 µCi/ml ^{14}C-cholesterol and 3µCi/ml ^{3}H-choline chloride. The medium was changed and during the chase period cells were incubated with or without HDL_3 for 12 hours. The ^{14}C-cholesterol and ^{3}H-choline content in the medium and in the cell lysate was measured and the efflux was determined in percent of total ^{14}C-cholesterol and ^{3}H-choline loading.

The most effective antisense oligonucleotide (AS Nr.2) inhibited cholesterol and phospholipids efflux relative to cells that were treated with control antisense (AS control). A dose dependent decrease in cholesterol efflux of 16,79% (5nmol AS) and 32,01% (10 nmol AS) could be shown, respectively.

Antisense incubation

To inhibit the induction of ABCG1 cells were treated with three different antisense oligonucleotides targeting ABCG1 or one scrambled control-antisense oligonucleotide during the AcLDL-incubation period.

Determination of cholesterol and phosphatidylcholine efflux from monocytes in dependency of antisense oligonucleotide treatment

To measure the efflux of cholesterol and phospholipids the cells were pulsed in addition to AcLDL-incubation with 1,5 μCi/ml ^{14}C-cholesterol and 3μCi/ml ^{3}H-choline chloride. The medium was changed and in chase period the cells were incubated with or without HDL_3 for 12 hours. Lipid extraction was performed according to the method of Bligh and Dyer [26]. The ^{14}C-cholesterol and ^{3}H-choline content in the medium and in the cell lysate was measured by liquid scintillation counting and the efflux was determined in percent of total ^{14}C-cholesterol and ^{3}H-choline loading as described [27]

Computer analyses

DNA and protein sequence analyses were conducted using programs provided by HUSAR, Heidelberg, Germany: http://genius.embnet.dkfz-heidelberg.de:8080.

Example 6

Complete cDNA sequence of the human ATP binding cassette transporter 1 (ABCA1 (ABC1)) and assessing the cholesterol sensitive regulation of ABCA1 mRNA expression

cDNA Cloning and Primary Protein Structure

We have cloned a 6880-bp cDNA containing the complete coding region of the human ABCA1 gene (Figure 8) The open reading frame of 6603 bp encodes a 2201-amino acid protein with a predicted molecular weight of 220 kDa. This protein displays a 94% identity on the amino acid level in an alignment with mouse ABCA1 and can therefore be considered as the human ortholog.

Tissue Distribution of ABCA1 mRNA Expression

In order to examine the tissue-specific expression of ABCA1 a multiple tissue RNA master blot containing poly A^+ RNA from 50 human tissues was carried out. Northern Blot analysis demonstrates the presence of a ABCA1 specific signal in all tissues. It is mostly prominent in adrenal gland, liver, lung, placenta and all fetal tissues examined so far (Table 1). The weakest signals are found in kidney, pancreas, pituitary gland, mammary gland and bone marrow.

Sterol Regulation of ABCA1 mRNA Expression

In order to determine the regulation of ABCA1 in monocytes/macrophages during cholesterol loading/depletion Northern Blot analysis was performed. The cloned 1000-bp DNA fragment derived from PCR amplification of RNA from five day differentiated monocytes with primers ABCA1 3622f (*CGTCAGCACTCTGATGATGGCCTG*-3') and ABCA1 4620r (*TCTCTGCTATCTCCAACCTCA*-3') was hybridized to Northern Blots containing RNA of differentially cultivated monocytes (figure 12) As can be seen in lanes one to five, the ABCA1 mRNA is increased during in vitro differentiation of freshly isolated monocytes until day five. Longer cultivation results in a total loss of

expression. When the cells were incubated in the presence of AcLDL to induce sterol loading (lanes 6-8) beginning at day four, a much stronger accumulation of mRNA can be detected in comparison to control cells (lanes 2-5). When these cells were cultured with HDL_3 as cholesterol acceptor for 12h, 24h and 48h (lanes 9-11) the ABCA1 signal significantly decreases with respect to control cells incubated in the absence of HDL_3 (lanes 12-14). Taken together, these results indicate that ABCA1 is a sterol-sensitive gene which is induced by cholesterol loading and downregulated by cholesterol depletion.

Cell culture.

Peripheral blood monocytes were isolated by leukapheresis and counterflow elutriation (19JBC). To obtain fractions containing >90% CD 14 positive mononuclear phagocytes, cells were pooled and cultured on plastic Petri dishes in macrophage SFM medium (Gibco BRL) containing 25 U/ml recombinant human M-CSF (Genzyme) for various times in 5% CO_2 in air at 37°C. The cells were incubated in the absence (differentiation control) or presence of AcLDL (100 μg/ml) to induce sterol loading. Following this incubation the cells were cultured in fresh medium supplemented with or without HDL_3 (100 μg/ml) for additional times in order to achieve cholesterol efflux from the cells to its acceptor HDL_3.

Preparation of RNA and Northern blot analysis.

Total cellular RNA was isolated from the cells by guanidium isothiocyanate lysis and CsCl centrifugation (Chirgwin). The RNA isolated was quantitated spectrophotometrically and 15 μg samples were separated on a 1.2% agarose-formaldehyde gel and transferred to a nylon membrane (Schleicher & Schüll). After crosslinking with UV-irradiation (Stratalinker model 1800, Stratagene), the membranes were hybridized with a 1000 bp DNA fragment derived from PCR amplification with primers ABCA1 3622f and ABCA1 4620r, stripped and subsequently hybridized with a human β-actin probe. In order to determine the tissue-specific expression of ABCA1 a multiple tissue RNA master blot containing

poly A^+ RNA from 50 human tissues was purchased from Clontech. The probes were radiolabeled with [γ-^{32}P]dCTP (Amersham) using the Oligolabeling kit from Pharmacia. Hybridization and washing conditions were performed follwoing the method described previously (Virca).

cDNA cloning of human ABCA1

Based on sequence information of mouse ABCA1 cDNA we designed primers for RT-PCR analysis in order to amplify the human ABCA1 (ABC1) cDNA. Approximately 1µg of RNA from five day differentiated mononuclear phagocytes was reverse transcribed in a 20 µl reaction using the RNA PCR Core Kit from Perkin Elmer. An aliquot of the cDNA was used in a 100 µl PCR reaction performed with Amplitaq Gold (Perkin Elmer) and the following primer combinations: (primer names indicate the position in the corresponding mouse cDNA sequence):

mABC1-144f (5´-CAAACATGTCAGCTGTTACTGGA-3´) and

mABC1-643r (5´-TAGCCTTGCAAA-AATACCTTCTG-3´),

mABC1-1221f (5´-GTTGGAAAGATTCTCTATACACCTG-3´) and

mABC1-1910r (5´-CGTCAGCACTCTGATGATGGCCTG-3´),

mABC1-3622f (5´-TCTCTGCTATCTCCAACCTCA-3´) and

mABC1-4620r (5´-ACGTCTTCACCAGGTAATCTGAA-3´),

mABC1-5056f (5´-CTATCTGTGTCATCTTTGCGATG-3´) and

mABC1-5857r (5´-CGCTTCCTCCTATAGATCTTGGT-3´),

mABC1-6093f (5´-AAGAGAGCATGTGGA-GTTCTTTG-3´) and

mABC1-7051r (5´-CCCTGTAATGGAATTGTGTTCTC-3´),

hABC1-540f (5´-AACCTTCTCTGGGTTCCTGTATC-3´) and

hABC1-1300r (5´-AGTTCCTGGAA-GGTCTTGTTCAC-3´),

hABC1-1831f (5´-GCTGACCCCTTTGAGGACATGCG-3´) and

hABC1-3701r (5´-ATAGGTCAGCTCATGCCCTATGT-3´),

hABC1-4532f (5´-GCTGCC-TCCTCCACAAAGAAAAC-3´) and

hABC1-5134r (5´-GCTTTGCTGACCCGCTCC-TGGATC-3´),

hABC1-5800f (5´-GAGGCCAGAATGACATCTTAGAA-3´) and

hABC1-6259r (5´-CTTGACAACACTTAGGGCACAAT-3´).

All PCR products were cloned into the pUC18 plasmid vector and the nucleotide sequences were determined on a Pharmacia ALFexpress sequencer using the dideoxy chain-termination method and fluorescent dye-labeled primers.

Example 7

Identification of the 5'end of ABCG1

We could partially prove the 5'-end of ABCG1 published by Chen [7] that differs from the 5'-end published by Croop [6] obtained from the mRNA of human monocytes/macrophages using a 5' RACE approach. In detail the sequence according to Chen et al. downstream of position 25 was in agreement with our own data. In contrast, our identified sequence differs from the one reported by Chen [7] and Croop [6] at a site upstream of position 25 (Chen [7]). The sequence SEQ ID NO: 32 shows the newly identified 5'-end followed by the sequence published by Chen [7] from position 25.

Molecular cloning and characterisation of the ABCG1 5'UTR

We identified several fragments by screening of a λ phage library which contained a total of app. 3 kb of the 5' UTR upstream sequence of the human ABCG1 gene. The

sequence that comprises the 5'UTR and part of exon 1 (described above) are given in SEQ ID NO: 54.

The promoter activity of this sequence was proven by luciferase reporter gene assays in transiently transfected CHO cells.

Putative transcription factor binding sites within the promoter region with the highest likelihood ratio for the matched sequence as deduced from the TransFac database, GFB, Braunschweig, Germany. Multiple binding sites for SP-1, AP-1, AP-2 and CCAAT-binding factor (C/EBP family) are present within the first 1 kb of the putative promoter region.

Additionally, a transcription factor binding site involved in the regulation of apolipoprotein B was identified.

Example 8

Characterization of the human ABCA8 full length cDNA

The putative ABCA8 coding sequence is app. 6.5 kb in size. We successfully cloned and sequenced a 1kb segment of the human ABCA8 cDNA that encodes the putative second nucleotide binding site of the mature polypeptide (the sequence is shown in the sequence listing). The nucleotide sequence exhibits a 73% homology with the known human ABCA1 (ABC1) cDNA sequence.

We identified an alternative transcript in the cloned 1 kb coding region which consists of a 72 bp segment (see sequence listing). Genomic analysis of this region revealed that the alternative sequence is identical with a complete intron suggesting that the alternative mRNA is generated by intron retention. The retained intron introduces a preterminal stop codon and thus may code for a truncated ABCA8 variant.

ABCA8 also shows a cholesterol sensitive regulation of the mRNA expression (Table 2).

Tissue expression of ABCA8 is shown in table 1.

Example 9

Characterisation of the regulation of ABC transporter during differentiation of keratinocytic cells (HaCaT)

Differentiation of epidermal keratinocytes is accompanied by the synthesis of specific lipids composed mainly of sphingolipids (SL), free fatty acids (FFA), cholesterol (CH), and cholesterol sulfate, all involved in the establishment of the epidermal permeability barrier. The skin and, in particular, the proliferating layer of the epidermis is one of the most active sites of lipid synthesis in the entire organism. Cholesterol synthesis in normal human epidermis is LDL-independent, and circulating cholesterol levels do not affect the cutaneous de novo cholesterol synthesis. Fully differentiated normal human keratinocytes lack LDL receptors or its expression is very low, whereas in the normal human epidermis only basal cells express LDL receptors.

During keratinocyte differentiation a shift from polar glycerophospholipids to neutral lipids (FFA, TG) and also a replacement of short chain FFA by long chain highly saturated FFA is observed. The most important lipids for the barrier function of the skin are sphingolipids that account for one third of the lipids in the cornified layer, and consist of a large ceramide fraction as a result of glucosylceramide degradation by intercellular glycosidases and de novo synthesis of ceramide .

Glucosylceramide is synthesized intracellulary and stored in lamellar bodies and glucosylceramide synthase expression was found up-regulated during the differentiation of human keratinocytes.

Cholesterol sulfate is formed by the action of cholesterol sulfotransferase during keratinocyte differentiation . Cholesterol sulfate and the degrading enzyme steroid sulfatase are present in all viable epidermal layers, with the highest levels in the stratum granulosum. The gradient of cholesterol sulfate content across the stratum corneum (from inner to outer layers), and progressive desulfation of cholesterol sulfate regulate cell cohesiveness and normal stratum corneum keratinization and desquamation, respectively. Cholesterol sulfate induces transglutaminase 1 and the coordinate regulation of both factors is essential for normal keratinization .

The final step in lipid barrier formation involves lamellar body secretion and the subsequent post-secretory processing of polar lipids into their nonpolar lipid products through the action of hydrolytic enzymes that are simultaneously released (β-glucocerebrosidase, phospholipases, steroid sulfatase, acid sphingomyelinase). Disruption of the permeability barrier results in an increased cholesterol, fatty acid, and ceramide synthesis in the underlying epidermis. It has been shown that mRNA levels for the key enzymes required for cholesterol, fatty acid, and ceramide synthesis increased rapidly after artificial barrier disruption .

Currently the lipid transport systems in keratinocytes are poorly characterized. Several fatty acid transport related proteins have been identified in keratinocytes: plasma membrane fatty acid transport proteins (FATP) and intracellular fatty acid binding proteins (FABPs), most of them exhibiting high affinity for essential fatty acids. The expression of epidermal FABPs is up-regulated in hyperproliferative and inflammatory skin diseases, during keratinocyte differentiation and barrier disruption

Based on our data on macrophages, we propose several ABC transporters as putative candidates for cellular lipid export in keratinocytes. We have examined the expression of all known ABC transporters during HaCaT cells differentiation. The human HaCaT cell line has a full epidermal differentiation capacity. Keratinocytes grown in

vitro as a monolayer at low calcium concentration (< 0.1 mM) can be differentiated by increasing calcium concentration in the culture medium (1-2 mM) . We cultured HaCaT cells as a monolayer in calcium-free RMPI (Gibco) medium mixed with standard Ham's F12 medium at a ratio 3:1 supplemented with 10% chelex-treated FCS, Penicillin and Streptomycin. The final concentration of calcium in above medium was 0.06 mM. When the cells reached confluence (usually on 5^{th} day of the culture), calcium concentration was enhanced up to the level of 1.2 mM. The cells were seeded at a density of $2x10^5/ cm^{2}$ in 60 mm culture dishes. The culture medium was replaced every two day and the cells were harvested after 24 h, 48h h, 4 d, 6 da, 8 d and 10 d in culture, respectively. Total RNA from HaCaT cells was isolated using the isothiocyanate/cesium chloride-ultracentrifugation method.

The expression of all known human ABC transporters was examined during HaCaT cell differentiation (24 h, 48 h, 4 d, 6 d, 8 d, 10d, respectively) using a semi-quantitative RT-PCR approach (Table 6). The primer sets were generated from the published sequences of the ABC-transporters. Primers specific for GAPDH were used as a control. As a marker of keratinocyte differentiation CGT (ceramide glucosyl transferase) gene expression was assessed. Three of the transporters examined, ABCB1 (MDR1), ABCB4 (MDR3), ABCD3 (PMP70), were not expressed. ABCC6 (MRP6), ABCA1 (ABC1),ABCD2 (ALDR and ABCB9 (est122234) were expressed at low levels (Table 6)

Most of the other transporters exhibited a biphasic expression pattern or were downregulated during keratinocyte differentiation. There was, however, a high expression of ABCG1 (ABC8), ABCA8 (new) and ABCC3 (MRP3) indicative for their involvement in terminal keratinocyte lipid secretion for cholesterol, FFAs and ceramide-backbone lipids.. The two peroxisomal ABC transporters, ABCD2 (ALDR) and ABCD1 (ALDP) that mediate the transport of very long chain fatty acids into peroxisomes were initially expressed at relatively low levels and subsequently downregulated during differentiation. This is in agreement with the replacement of

short chain fatty acids by very long chain fatty acids during keratinocyte differentiation.

Example 10:

Sequencing of ABCA1 cDNA and genomic structure in five families of patients with Tangier disease revealed different mutations in the ABCA1 gene locus. These patients have different mutations at different positions in the ABCA1 gene, that result in changes in the protein structure of ABCA1. Family members that are heterozygous for these mutations show lowered levels of serum HDL, whereas the homocygote patients have extremely reduced HDL serum levels.

Claims:

1. A polynucleotide comprising a member selected from the group consisting of:

   (a) a polynucleotide encoding the polypeptide as set forth in SEQ ID NO:2;
   (b) a polynucleotide capable of hybridizing to and which is at least 70% identical to the polynucleotide of (a); and
   (c) a polynucleotide fragment of the polynucleotide of (a) or (b).

2. The polynucleotide of claim 1 wherein the polynucleotide is DNA.

3. A vector containing one or more of the polynucleotides of claim 1 and 2.

4. A host cell containing the vector of claim 3.

5. A process for producing a polypeptide comprising: expressing from the host cell of claim 4 the polypeptide encoded by said DNA.

6. A polypeptide selected from the group consisting of

   (a) a polypeptide having the deduced amino acid sequence of SEQ ID NO:2 and fragments, analogs and derivatives thereof, and
   (b) a polypeptide comprising amino acid 1 to amino acid 2201 of SEQ ID NO:2.

7. An antibody capable to bind to the polypeptide of claim 6.

8. A diagnostic kit for the detection of the polypeptide of claim 6.

9. Use of a polypeptides encoded by a polynucleotide comprising a member selected from the group consisting of:

   (a) a polynucleotide as set forth in SEQ ID NO:1, 3, 4 and 6 to 31;
   (b) a polynucleotide capable of hybridizing to and which is at least 70% identical to the polynucleotide of (a); and
   (c) a polynucleotide fragment of the polynucleotide of (a) or (b)

   in an assay for for detecting modulators of said polypeptides.

10. Modulator of a polypeptides encoded by a polynucleotide comprising a member selected from the group consisting of:

   (a) a polynucleotide as set forth in SEQ ID NO:1, 3, 4 and 6 to 31;
   (b) a polynucleotide capable of hybridizing to and which is at least 70% identical to the polynucleotide of (a); and
   (d) a polynucleotide fragment of the polynucleotide of (a) or (b)

11. A pharmaceutical comprising the modulator of claim 10

12. An assay for detecting polypeptides encoded by a polynucleotide comprising a member selected from the group consisting of:

   (a) a polynucleotide as set forth in SEQ ID NO:1, 3, 4 and 6 to 32 and 54;
   (b) a polynucleotide capable of hybridizing to and which is at least 70% identical to the polynucleotide of (a); and
   (c) a polynucleotide fragment of the polynucleotide of (a) or (b)

Figure 1

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     2588 GA TCAATCGCAT TCATTTTAAG AAATTATACC TTTTTAGTAC TTGCTGAAGA
2641 ATGATTCAGG GTAAATCACA TACTTTGTTT AGAGAGGCGA GGGGTTTAAC CCGAGTCACC
2701 CAGCTGGTCT CATACATAGA CAGCACTTGT GAAGGATTGA ATGCAGGTTC CAGGTGGAGG
2761 GAAGACGTGG ACACCATCTC CACTGAGCCA TGCAGACATT TTTAAAAGCT ATACACAAAA
2821 TTGTGAGAAG ACATTGGCCA ACTCTTTCAA AGTCTTTCTT TTTCCACGTG CTTCTTATTT
2881 TAAGCGAAAT ATATTGTTTG TTTCTTCCTA AAAAAAAAAA 2890
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Figure 2

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  1 CAAACATGTCAGCTGTTACTGGAAGTGGCCTGGCCTCTATTTATCTTCCTGATCCTGATC 60
 61 TCTGTTCGGCTGAGCTACCCACCCTATGAACAACATGAATGCCATTTTCCAAATAAAGCC 120
121 ATGCCCTCTGCAGGAACACTTCCTTGGGTTCAGGGGATTATCTGTAATGCCAACAACCCC 180
  1 M P S A G T L P W V Q G I I C N A N N P 20
181 TGTTTCCGTTACCCGACTCCTGGGGAGGCTCCCGGAGTTGTTGGAAACTTTAACAAATCC 240
 21 C F R Y P T P G E A P G V V G N F N K S 40
241 ATTGTGGCTCGCCTGTTCTCAGATGCTCGGAGGCTTCTTTTATACAGCCAGAAAGACACC 300
 41 I V A R L F S D A R R L L L Y S Q K D T 60
301 AGCATGAAGGACATGCGCAAAGTTCTGAGAACATTACAGCAGATCAAGAAATCCAGCTCA 360
 61 S M K D M R K V L R T L Q Q I K K S S S 80
361 AACTTGAAGCTTCAAGATTTCCTGGTGGACAATGAAACCTTCTCTGGGTTCCTGTATCAC 420
 81 N L K L Q D F L V D N E T F S G F L Y H 100
421 AACCTCTCTCTCCCAAAGTCTACTGTGGACAAGATGCTGAGGGCTGATGTCATTCTCCAC 480
101 N L S L P K S T V D K M L R A D V I L H 120
481 AAGGTATTTTTGCAAGGCTACCAGTTACATTTGACAAGTCTGTGCAATGGATCAAAATCA 540
121 K V F L Q G Y Q L H L T S L C N G S K S 140
541 GAAGAGATGATTCAACTTGGTGACCAAGAAGTTTCTGAGCTTTGTGGCCTACCAAGGGAG 600
141 E E M I Q L G D Q E V S E L C G L P R E 160
601 AAACTGGCTGCAGCAGAGCGAGTACTTCGTTCCAACATGGACATCCTGAAGCCAATCCTG 660
161 K L A A A E R V L R S N M D I L K P I L 180
661 AGAACACTAAACTCTACATCTCCCTTCCCGAGCAAGGAGCTGGCCGAAGCCACAAAAACA 720
181 R T L N S T S P F P S K E L A E A T K T 200
721 TTGCTGCATAGTCTTGGGACTCTGGCCCAGGAGCTGTTCAGCATGAGAAGCTGGAGTGAC 780
201 L L H S L G T L A Q E L F S M R S W S D 220
781 ATGCGACAGGAGGTGATGTTTCTGACCAATGTGAACAGCTCCAGCTCCTCCACCCAAATC 840
221 M R Q E V M F L T N V N S S S S S T Q I 240
841 TACCAGGCTGTGTCTCGTATTGTCTGCGGGCATCCCGAGGGAGGGGGGCTGAAGATCAAG 900
241 Y Q A V S R I V C G H P E G G G L K I K 260
901 TCTCTCAACTGGTATGAGGACAACAACTACAAAGCCCTCTTTGGAGGCAATGGCACTGAG 960
261 S L N W Y E D N N Y K A L F G G N G T E 280
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961 GAAGATGCTGAAACCTTCTATGACAACTCTACAACTCCTTACTGCAATGATTTGATGAAG 1020
281 E D A E T F Y D N S T T P Y C N D L M K 300
1021 AATTTGGAGTCTAGTCCTCTTTCCCGCATTATCTGGAAAGCTCTGAAGCCGCTGCTCGTT 1080
301 N L E S S P L S R I I W K A L K P L L V 320
1081 GGGAAGATCCTGTATACACCTGACACTCCAGCCACAAGGCAGGTCATGGCTGAGGTGAAC 1140
321 G K I L Y T P D T P A T R Q V M A E V N 340
1141 AAGACCTTCCAGGAACTGGCTGTGTTCCATGATCTGGAAGGCATGTGGGAGGAACTCAGC 1200
341 K T F Q E L A V F H D L E G M W E E L S 360
1201 CCCAAGATCTGGACCTTCATGGAGAACAGCCAAGAAATGGACCTTGTCCGGATGCTGTTG 1260
361 P K I W T F M E N S Q E M D L V R M L L 380
1261 GACAGCAGGGACAATGACCACTTTTGGGAACAGCAGTTGGATGGCTTAGATTGGACAGCC 1320
381 D S R D N D H F W E Q Q L D G L D W T A 400
1321 CAAGACATCGTGGCGTTTTTGGCCAAGCACCCAGAGGATGTCCAGTCCAGTAATGGTTCT 1380
401 Q D I V A F L A K H P E D V Q S S N G S 420
1381 GTGTACACCTGGAGAGAAGCTTTCAACGAGACTAACCAGGCAATCCGGACCATATCTCGC 1440
421 V Y T W R E A F N E T N Q A I R T I S R 440
1441 TTCATGGAGTGTGTCAACCTGAACAAGCTAGAACCCATAGCAACAGAAGTCTGGCTCATC 1500
441 F M E C V N L N K L E P I A T E V W L I 460
1501 AACAAGTCCATGGAGCTGCTGGATGAGAGGAAGTTCTGGGCTGGTATTGTGTTCACTGGA 1560
461 N K S M E L L D E R K F W A G I V F T G 480
1561 ATTACTCCAGGCAGCATTGAGCTGCCCCATCATGTCAAGTACAAGATCCGAATGGACATT 1620
481 I T P G S I E L P H H V K Y K I R M D I 500
1621 GACAATGTGGAGAGGACAAATAAAATCAAGGATGGGTACTGGGACCCTGGTCCTCGAGCT 1680
501 D N V E R T N K I K D G Y W D P G P R A 520
1681 GACCCCTTTGAGGACATGCGGTACGTCTGGGGGGGCTTCGCCTACTTGCAGGATGTGGTG 1740
521 D P F E D M R Y V W G G F A Y L Q D V V 540
1741 GAGCAGGCAATCATCAGGGTGCTGACGGGCACCGAGAAGAAAACTGGTGTCTATATGCAA 1800
541 E Q A I I R V L T G T E K K T G V Y M Q 560
1801 CAGATGCCCTATCCCTGTTACGTTGATGACATCTTTCTGCGGGTGATGAGCCGGTCAATG 1860
561 Q M P Y P C Y V D D I F L R V M S R S M 580
1861 CCCCTCTTCATGACGCTGGCCTGGATTTACTCAGTGGCTGTGATCATCAAGGGCATCGTG 1920
581 P L F M T L A W I Y S V A V I I K G I V 600
1921 TATGAGAAGGAGGCACGGCTGAAAGAGACCATGCGGATCATGGGCCTGGACAACAGCATC 1980
601 Y E K E A R L K E T M R I M G L D N S I 620
1981 CTCTGGTTTAGCTGGTTCATTAGTAGCCTCATTCCTCTTCTTGTGAGCGCTGGCCTGCTA 2040
621 L W F S W F I S S L I P L L V S A G L L 640
2041 GTGGTCATCCTGAAGTTAGGAAACCTGCTGCCCTACAGTGATCCCAGCGTGGTGTTTGTC 2100
641 V V I L K L G N L L P Y S D P S V V F V 660

2101 TTCCTGTCCGTGTTTGCTGTGGTGACAATCCTGCAGTGCTTCCTGATTAGCACACTCTTC 2160

661 F L S V F A V V T I L Q C F L I S T L F 680
2161 TCCAGAGCCAACCTGGCAGCAGCCTGTGGGGGCATCATCTACTTCACGCTGTACCTGCCC 2220
681 S R A N L A A A C G G I I Y F T L Y L P 700
2221 TACGTCCTGTGTGTGGCATGGCAGGACTACGTGGGCTTCACACTCAAGATCTTCGCTAGC 2280
701 Y V L C V A W Q D Y V G F T L K I F A S 720
2281 CTGCTGTCTCCTGTGGCTTTTGGGTTTGGCTGTGAGTACTTTGCCCTTTTTGAGGAGCAG 2340
721 L L S P V A F G F G C E Y F A L F E E Q 740
2341 GGCATTGGAGTGCAGTGGGACAACCTGTTTGAGAGTCCTGTGGAGGAAGATGGCTTCAAT 2400
741 G I G V Q W D N L F E S P V E E D G F N 760
2401 CTCACCACTTCGGTCTCCATGATGCTGTTTGACACCTTCCTCTATGGGGTGATGACCTGG 2460
761 L T T S V S M M L F D T F L Y G V M T W 780
2461 TACATTGAGGCTGTCTTTCCAGGCCAGTACGGAATTCCCAGGCCCTGGTATTTTCCTTGC 2520
781 Y I E A V F P G Q Y G I P R P W Y F P C 800
2521 ACCAAGTCCTACTGGTTTGGCGAGGAAAGTGATGAGAAGAGCCACCCTGGTTCCAACCAG 2580
801 T K S Y W F G E E S D E K S H P G S N Q 820
2581 AAGAGAATATCAGAAATCTGCATGGAGGAGGAACCCACCCACTTGAAGCTGGGCGTGTCC 2640
821 K R I S E I C M E E E P T H L K L G V S 840
2641 ATTCAGAACCTGGTAAAAGTCTACCGAGATGGGATGAAGGTGGCTGTCGATGGCCTGGCA 2700
841 I Q N L V K V Y R D G M K V A V D G L A 860
2701 CTGAATTTTTATGAGGGCCAGATCACCTCCTTCCTGGGCCACAATGGAGCGGGGAAGACG 2760
861 L N F Y E G Q I T S F L G H N G A G K T 880
2761 ACCACCATGTCAATCCTGACCGGGTTGTTCCCCCCGACCTCGGGCACCGCCTACATCCTG 2820
881 T T M S I L T G L F P P T S G T A Y I L 900
2821 GGAAAAGACATTCGCTCTGAGATGAGCACCATCCGGCAGAACCTGGGGGTCTGTCCCCAG 2880
901 G K D I R S E M S T I R Q N L G V C P Q 920
2881 CATAACGTGCTGTTTGACATGCTGACTGTCGAAGAACACATCTGGTTCTATGCCCGCTTG 2940
921 H N V L F D M L T V E E H I W F Y A R L 940
2941 AAAGGGCTCTCTGAGAAGCACGTGAAGGCGGAGATGGAGCAGATGGCCCTGGATGTTGGT 3000
941 K G L S E K H V K A E M E Q M A L D V G 960
3001 TTGCCATCAAGCAAGCTGAAAAGCAAAACAAGCCAGCTGTCAGGTGGAATGCAGAGAAAG 3060
961 L P S S K L K S K T S Q L S G G M Q R K 980
3061 CTATCTGTGGCCTTGGCCTTTGTCGGGGGATCTAAGGTTGTCATTCTGGATGAACCCACA 3120
981 L S V A L A F V G G S K V V I L D E P T 1000
3121 GCTGGTGTGGACCCTTACTCCCGCAGGGGAATATGGGAGCTGCTGCTGAAATACCGACAA 3180
1001 A G V D P Y S R R G I W E L L L K Y R Q 1020
3181 GGCCGCACCATTATTCTCTCTACACACCACATGGATGAAGCGGACGTCCTGGGGGACAGG 3240
1021 G R T I I L S T H H M D E A D V L G D R 1040
3241 ATTGCCATCATCTCCCATGGGAAGCTGTGCTGTGTGGGCTCCTCCCTGTTTCTGAAGAAC 3300
1041 I A I I S H G K L C C V G S S L F L K N 1060
3301 CAGCTGGGAACAGGCTACTACCTGACCTTGGTCAAGAAAGATGTGGAATCCTCCCTCAGT 3360

1061 Q L G T G Y Y L T L V K K D V E S S L S 1080
3361 TCCTGCAGAAACAGTAGTAGCACTGTGTCATACCTGAAAAAGGAGGACAGTGTTTCTCAG 3420
1081 S C R N S S S T V S Y L K K E D S V S Q 1100
3421 AGCAGTTCTGATGCTGGCCTGGGCAGCGACCATGAGAGTGACACGCTGACCATCGATGTC 3480
1101 S S S D A G L G S D H E S D T L T I D V 1120
3481 TCTGCTATCTCCAACCTCATCAGGAAGCATGTGTCTGAAGCCCGGCTGGTGGAAGACATA 3540
1121 S A I S N L I R K H V S E A R L V E D I 1140
3541 GGGCATGAGCTGACCTATGTGCTGCCATATGAAGCTGCTAAGGAGGGAGCCTTTGTGGAA 3600
1141 G H E L T Y V L P Y E A A K E G A F V E 1160
3601 CTCTTTCATGAGATTGATGACCGGCTCTCAGACCTGGGCATTTCTAGTTATGGCATCTCA 3660
1161 L F H E I D D R L S D L G I S S Y G I S 1180
3661 GAGACGACCCTGGAAGAAATATTCCTCAAGGTGGCCGAAGAGAGTGGGGTGGATGCTGAG 3720
1181 E T T L E E I F L K V A E E S G V D A E 1200
3721 ACCTCAGATGGTACCTTGCCAGCAAGACGAAACAGGCGGGCCTTCGGGGACAAGCAGAGC 3780
1201 T S D G T L P A R R N R R A F G D K Q S 1220
3781 TGTCTTCGCCCGTTCACTGAAGATGATGCTGCTGATCCAAATGATTCTGACATAGACCCA 3840
1221 C L R P F T E D D A A D P N D S D I D P 1240
3841 GAATCCAGAGAGACAGACTTGCTCAGTGGGATGGATGGCAAAGGGTCCTACCAGGTGAAA 3900
1241 E S R E T D L L S G M D G K G S Y Q V K 1260
3901 GGCTGGAAACTTACACAGCAACAGTTTGTGGCCCTTTTGTGGAAGAGACTGCTAATTGCC 3960
1261 G W K L T Q Q Q F V A L L W K R L L I A 1280
3961 AGACGGAGTCGGAAAGGATTTTTTGCTCAGATTGTCTTGCCAGCTGTGTTTGTCTGCATT 4020
1281 R R S R K G F F A Q I V L P A V F V C I 1300
4021 GCCCTTGTGTTCAGCCTGATCGTGCCACCCTTTGGCAAGTACCCCAGCCTGGAACTTCAG 4080
1301 A L V F S L I V P P F G K Y P S L E L Q 1320
4081 CCCTGGATGTACAACGAACAGTACACATTTGTCAGCAATGATGCTCCTGAGGACACGGGA 4140
1321 P W M Y N E Q Y T F V S N D A P E D T G 1340
4141 ACCCTGGAACTCTTAAACGCCCTCACCAAAGACCCTGGCTTCGGGACCCGCTGTATGGAA 4200
1341 T L E L L N A L T K D P G F G T R C M E 1360
4201 GGAAACCCAATCCCAGACACGCCCTGCCAGGCAGGGGAGGAAGAGTGGACCACTGCCCCA 4260
1361 G N P I P D T P C Q A G E E E W T T A P 1380
4261 GTTCCCCAGACCATCATGGACCTCTTCCAGAATGGGAACTGGACAATGCAGAACCCTTCA 4320
1381 V P Q T I M D L F Q N G N W T M Q N P S 1400
4321 CCTGCATGCCAGTGTAGCAGCGACAAAATCAAGAAGATGCTGCCTGTGTGTCCCCCAGGG 4380
1401 P A C Q C S S D K I K K M L P V C P P G 1420
4381 GCAGGGGGGCTGCCTCCTCCACAAAGAAAACAAAACACTGCAGATATCCTTCAGGACCTG 4440
1421 A G G L P P P Q R K Q N T A D I L Q D L 1440
4441 ACAGGAAGAAACATTTCGGATTATCTGGTGAAGACGTATGTGCAGATCATAGCCAAAAGC 4500
1441 T G R N I S D Y L V K T Y V Q I I A K S 1460
4501 TTAAAGAACAAGATCTGGGTGAATGAGTTTAGGTATGGCGGCTTTTCCCTGGGTGTCAGT 4560

1461 L K N K I W V N E F R Y G G F S L G V S 1480
4561 AATACTCAAGCACTTCCTCCGAGTCAAGAAGTTAATGATGCCACCAAACAAATGAAGAAA 4620
1481 N T Q A L P P S Q E V N D A T K Q M K K 1500
4621 CACCTAAAGCTGGCCAAGGACAGTTCTGCAGATCGATTTCTCAACAGCTTGGGAAGATTT 4680
1501 H L K L A K D S S A D R F L N S L G R F 1520
4681 ATGACAGGACTGGACACCAGAAATAATGTCAAGGTGTGGTTCAATAACAAGGGCTGGCAT 4770
1521 M T G L D T R N N V K V W F N N K G W H 1540
4741 GCAATCAGCTCTTTCCTGAATGTCATCAACAATGCCATTCTCCGGGCCAACCTGCAAAAG 4800
1541 A I S S F L N V I N N A I L R A N L Q K 1560
4801 GGAGAGAACCCTAGCCATTATGGAATTACTGCTTTCAATCATCCCCTGAATCTCACCAAG 4860
1561 G E N P S H Y G I T A F N H P L N L T K 1580
4861 CAGCAGCTCTCAGAGGTGGCTCCGATGACCACATCAGTGGATGTCCTTGTGTCCATCTGT 4920
1581 Q Q L S E V A P M T T S V D V L V S I C 1600
4921 GTCATCTTTGCAATGTCCTTCGTCCCAGCCAGCTTTGTCGTATTCCTGATCCAGGAGCGG 4980
1601 V I F A M S F V P A S F V V F L I Q E R 1620
4981 GTCAGCAAAGCAAAACACCTGCAGTTCATCAGTGGAGTGAAGCCTGTCATCTACTGGCTC 5040
1621 V S K A K H L Q F I S G V K P V I Y W L 1640
5041 TCTAATTTTGTCTGGGATATGTGCAATTACGTTGTCCCTGCCACACTGGTCATTATCATC 5100
1641 S N F V W D M C N Y V V P A T L V I I I 1660
5101 TTCATCTGCTTCCAGCAGAAGTCCTATGTGTCCTCCACCAATCTGCCTGTGCTAGCCCTT 5160
1661 F I C F Q Q K S Y V S S T N L P V L A L 1680
5161 CTACTTTTGCTGTATGGGTGGTCAATCACACCTCTCATGTACCCAGCCTCCTTTGTGTTC 5220
1681 L L L L Y G W S I T P L M Y P A S F V F 1700
5221 AAGATCCCCAGCACAGCCTATGTGGTGCTCACCAGCGTGAACCTCTTCATTGGCATTAAT 5280
1701 K I P S T A Y V V L T S V N L F I G I N 1720
5281 GGCAGCGTGGCCACCTTTGTGCTGGAGCTGTTCACCGACAATAAGCTGAATAATATCAAT 5340
1721 G S V A T F V L E L F T D N K L N N I N 1740
5341 GATATCCTGAAGTCCGTGTTCTTGATCTTCCCACATTTTTGCCTGGGACGAGGGCTCATC 5400
1741 D I L K S V F L I F P H F C L G R G L I 1760
5401 GACATGGTGAAAAACCAGGCAATGGCTGATGCCCTGGAAAGGTTTGGGGAGAATCGCTTT 5460
1761 D M V K N Q A M A D A L E R F G E N R F 1780
5461 GTGTCACCATTATCTTGGGACTTGGTGGGACGAAACCTCTTCGCCATGGCCGTGGAAGGG 5520
1781 V S P L S W D L V G R N L F A M A V E G 1800
5521 GTGGTGTTCTTCCTCATTACTGTTCTGATCCAGTACAGATTCTTCATCAGGCCCAGACCT 5580
1801 V V F F L I T V L I Q Y R F F I R P R P 1820
5581 GTAAATGCAAAGCTATCTCCTCTGAATGATGAAGATGAAGATGTGAGGCGGGAAAGACAG 5640
1821 V N A K L S P L N D E D E D V R R E R Q 1840
5641 AGAATTCTTGATGGTGGAGGCCAGAATGACATCTTAGAAATCAAGGAGTTGACGAAGATA 5700
1841 R I L D G G G Q N D I L E I K E L T K I 1860
5701 TATAGAAGGAAGCGGAAGCCTGCTGTTGACAGGATTTGCGTGGGCATTCCTCCTGGTGAG 5760

1861 Y R R K R K P A V D R I C V G I P P G E 1880

5761 TGCTTTGGGCTCCTGGGAGTTAATGGGGCTGGAAAATCATCAACTTTCAAGATGTTAACA 5820

1881 C F G L L G V N G A G K S S T F K M L T 1900

5821 GGAGATACCACTGTTACCAGAGGAGATGCTTTCCTTAACAGAAATAGTATCTTATCAAAC 5880

1901 G D T T V T R G D A F L N R N S I L S N 1920

5881 ATCCATGAAGTACATCAGAACATGGGCTACTGCCCTCAGTTTGATGCCATCACAGAGCTG 5940

1921 I H E V H Q N M G Y C P Q F D A I T E L 1940

5941 TTGACTGGGAGAGAACACGTGGAGTTCTTTGCCCTTTTGAGAGGAGTCCCAGAGAAAGAA 6000

1941 L T G R E H V E F F A L L R G V P E K E 1960

6001 GTTGGCAAGGTTGGTGAGTGGGCGATTCGGAAACTGGGCCTCGTGAAGTATGGAGAAAAA 6060

1961 V G K V G E W A I R K L G L V K Y G E K 1980

6061 TATGCTGGTAACTATAGTGGAGGCAACAAACGCAAGCTCTCTACAGCCATGGCTTTGATC 6120

1981 Y A G N Y S G G N K R K L S T A M A L I 2000

6121 GGCGGGCCTCCTGTGGTGTTTCTGGATGAACCCACCACAGGCATGGATCCCAAAGCCCGG 6180

2001 G G P P V V F L D E P T T G M D P K A R 2020

6181 CGGTTCTTGTGGAATTGTGCCCTAAGTGTTGTCAAGGAGGGGAGATCAGTAGTGCTTACA 6240

2021 R F L W N C A L S V V K E G R S V V L T 2040

6241 TCTCATAGTATGGAAGAATGTGAAGCTCTTTGCACTAGGATGGCAATCATGGTCAATGGA 6300

2041 S H S M E E C E A L C T R M A I M V N G 2060

6301 AGGTTCAGGTGCCTTGGCAGTGTCCAGCATCTAAAAAATAGGTTTGGAGATGGTTATACA 6360

2061 R F R C L G S V Q H L K N R F G D G Y T 2080

6361 ATAGTTGTACGAATAGCAGGGTCCAACCCGGACCTGAAGCCTGTCCAGGATTTCTTTGGA 6420

2081 I V V R I A G S N P D L K P V Q D F F G 2100

6421 CTTGCATTTCCTGGAAGTGTTCCAAAAGAGAAACACCGGAACATGCTACAATACCAGCTT 6480

2101 L A F P G S V P K E K H R N M L Q Y Q L 2120

6481 CCATCTTCATTATCTTCTCTGGCCAGGATATTCAGCATCCTCTCCCAGAGCAAAAAGCGA 6540

2121 P S S L S S L A R I F S I L S Q S K K R 2140

6541 CTCCACATAGAAGACTACTCTGTTTCTCAGACAACACTTGACCAAGTATTTGTGAACTTT 6600

2141 L H I E D Y S V S Q T T L D Q V F V N F 2160

6601 GCCAAGGACCAAAGTGATGATGACCACTTAAAAGACCTCTCATTACACAAAAACCAGACA 6660

2161 A K D Q S D D D H L K D L S L H K N Q T 2180

6661 GTAGTGGACGTTGCAGTTCTCACATCTTTTCTACAGGATGAGAAAGTGAAAGAAAGCTAT 6720

2181 V V D V A V L T S F L Q D E K V K E S Y 2200

6721 GTATGAAGAATCCTGTTCATACGGGGTGGCTGAAAGTAAAGAGGGACTAGACTTTCCTTT 6780

2201 V *

6781 GCACCATGTGAAGTGTTGTGGAGAAAAGAGCCAGAAGTTGATGTGGGAAGAAGTAAACTG 6840

6841 GATACTGTACTGATACTATTCAATGCAATGCAATTCAATG 6880

Figure 3

5′ 1 GTACCCCCCT TGCCTGGTTG ATCCTCAGGG TTCTACTTAG AATGCCTCGA

51 AAAGTCTTGG CTGGACACCC ATGCCCAGTC TTTCTGCAGG GTCCCATTGG
101 GGTTAACCTT CTCATTTCAT CCCATGTGAA CCAGGCCAGG CCCATCAGGG
151 TTTGGCAACC CCCTGATGCA GTGGTTGCTG CCAGGTGACA GGAGCAAGCC
201 TGCAGCTGCT GGGGGGCCAT GCAGAGACAG CCTGCCAGAG GGGAGACCAC
251 CTGGGGAGGC CAGAGCCGTG GAGACAGCAA GAGACCAGGG GCTGAGGACA
301 GAGTAGTACA GGTCTTTGGT CCCAGTAGTC CTGAAACCAC TGCACTCCGA
351 ACCTTTCTGT ACTTAGCTTA AGCCAGTTGG AGTTTCTGTC CTTTACAACC
401 AAGAGCCTTG ATAGGAATGG GGTCCTGTGC TACGCTACTG TTGGCTTCTT
451 TCCCGATCGG GCGCTGGAGG GGAACACAGC AGTGACTACA GTGGGATGCT
501 TACTCGGTGC TGGGCATGCT AGAAAGTGCT TGCCATGCCT TATTTCCCAC
551 GTGGTGGGGA TTTTGACCCC ACCTGTACAG ACAGATAAGT GAGGACCCTT
601 TTCACCTTAT CCTGCAACAG AAAATCCAGC AGCCAAAGCC AACAAGGGCC
651 CAGCATAGCA TCTTCCCTCT CTGACTTCAT CCTCACGCTC CACACACCAT
701 CCCCCTGGCC ATTCCCAGCA GCCCAGTAAG CACTGCCTCA CACTTCCAGT
751 TCCGGACCAG CCAGGATGGC CAGGCTGGAT GGGGGCCATC CACCGGCTGA
801 AGCCAATTGC CTATTCTCGA GCTGAAGGTG AATCAATCCC GCATAAATCT
851 TCGGGCAGAG AACTNGGGTG GGGGGTAGAA GAGGGGGAAT GTCTAGAAGG
901 AAATTCTGGG GCACATTCCT GGAAGTGAGG AGGATGGATA TTGGACAGAA
951 ATTATGTCAT TGCAGGCACC CTCACTTGCC CTGGCCACAT GGACAGTTCC
1001 TCCCCGGCTG TGTTCCGNGC CTCCTCTCGT GCTCCAGGGC CTGTCTGTTC
1051 CTGGAGCGAG ATGGGTCCCA GGGCTGGGCA CCAGTCCCCA TCTCCAGCCA
1101 TCAGGCACTT TCCTCTCTGT GTTTTGGCGT AAACACNTCC CTAGGTTTGT
1151 GGATCTGAAT CCTCTTCCCA ACACACTCAA GCTTTGCTGG GCCTCCCTGC
1201 AGTGTATGTT TAAGGCACCA CACAGCCTCC AAGGCCTGGC ACCCGGGCAG
1251 TGGCCACCTG GTAAACACAG CAGTCAGATT TCCTCATTTC AGCCAAGTGT
1301 AAAATCAAGG TAATGGATCT ACNCTTTTTT TTTTNTNTTT TTTCCAGGGG
1351 GNTNNTTTTT TTTTGAGACG GAGTCTCACT CTGTCANCCC CGGTCTGGAG
1401 TGCAGTGGCT CAATCTCGGC TCANCTGGCA AGCTCCGCCT CCCAGGTTCA
1451 TGCCATTCTC CTGCCTCAGC CTACATAGTA GCTGGGACTA CAGGTGCCCG
1501 CCACCACACC TAGCTAATTT TTTGTATTTT TAGTAGAGAC GGGGTTTCAT
1551 CATGTTAGCC AGGATGGTCT CGATCTCCTG ACCTCCCAAA GTGGTGGGAG
1601 TTACAGGTGT GAGCCACTGC GCNCCGGCTG GATGACTCTT GAGACAACAC
1651 CATTCAGACA AAGGCAAGGC CTCCCACTTA AACTCATAAC CGTGTCTCCT
1701 TTCTCTCCTT CGATTTGAGC GGCTGAATTT GGTTACAGTC ATCTGACCTG
1751 TGGGTGTGAA NGTCCACCTG CCTGGCATAA AAAGCTGTGC CTCCTTTCTA
1801 GGTGAGGAGA AAGAGAGAGA CCTGGCTCAT CTGAGGTGTG GTTGGGAGGG
1851 GGGACCCAGG TGTGCTGGAA ATGAAAAGAA ATGCATTCCT GTTTTTTCGT
1901 CCCAACATGC AAACAACTGA ACAAAAGCAT TAGGGCCTGA GACTGGGAGT
1951 AAAGAATTCC TTGTCACCAT GGATACCAGG AAATGGCCCC ACTTATATAT
2001 AATAAGGGCT TTAGAGATGC TGGACCATCT GATATTCCAG CCTGGGGCCA
2051 CATGGGAGTG TGCCCTGGTG TTATTCCTTA TACAGTTCCA TGAACATGGC
2101 TCTGGAAACA CCTCTGTCTG CAGAAAATGA GGCTTTTCTT TTTTTGTTCG

2151 GGGGTGAACA GAGGGCAGAG GCCTGGGCAT CTTCACTCAG CACCCCTTTG
2201 TAACCCAGCA CTTAGCACCA TGGCTGGCGC ACAGCAATGT CACATGTGTG
2251 AGTGCACACG ATGCCTCACT GCCAGGGGTC ACCCCACACC GGTGCTGTTG
2301 GGGGCGTTGG AGTGGTTATC TCTTCTTTAG TCCTCAAGCT CCTACCTGGC
2351 AGAGAGCTGC CCAACACCGT CGGGGTGGGG TGGGCGGGAA GGGAAGAAGC
2401 AGCAGCAAGA AAGAAGCCCC CTGGCCCTCA CTCTCCCTCC CTGGACGCCC
2451 CCTCTTCGAC CCCATCACAC AGCCGCTTGA GCCTTGGAGN CAGTGGATTT
2501 CCGAGCCTGG GAACCCCCGG CGTCTGTCCC GGTGTCCCCC GCAGCCTCAC
2551 CCNCGTGCTG GCCCAGCCCC CGCGAGTTCG GGACCCGGGG TTTCCGGGGT
2601 GGCAGGGGGT TCCCATGCCG CCTGCGAGGC CTCGGCTCGG GCCGCTCCCG
2651 GAACCTGCAC TTCAGGGGTC CTGGTCCGCC GCCCCCAGCA GGAGCAAAAC
2701 AAGAGCACGC GCACCTGCCG GCCCGCCCGC CCCCTTGGTG CCGGCCAATC
2751 GCGCGCTCGG GGCGGGGTCG GGCGCGCTGG AACCAGAGCC GGAGCCGGAT
2801 CCCAGCCGGA GCCCAAGCGC AGCCCGCACC CCGCGCAGCG GCTGAGCCGG
2851 GAGCCAGCGC AGCCTCGGCC CCGCAGCTCA AGCCTCGTCC CCGCCGCCNG
2901 CCGCCGCACG CCGCCGCCGC CGCCCCCGGG GC**ATG**GCTGT CTGATGGCCG

EXON1/INTRON 1

2951 CTTTCTCGGT CGGCACCGCC AT**GG**TGAGTG AGCGCATCCT TCGTCCGCCG
3001 GGAACGGTTT TATTTTCAAG GAGAGCAGGA AACACACAAA GACTCGCAAG
3051 CTCGACCTGA CACCCCTCCC AGGAGCGCGT CCTCTGGGGC GCTGACCCAG
3101 GGGCACCCTA GAGTGGCGCC CGGCTCCGAT CGCTGCCCCT NNCCCCTCCG
3151 CCAGGGCCAC CTGGGAGCCT CGGGGATGCC CCTTGCACCG GCAGAGNGCA
3201 CGGACTAGGT GGAGGGGNCC GGGATTGGGG CGGGGGGCAG NCAGTTGCCC
3251 TACAAGTTGG ACCGATGGCC TTGACCTGAT GGCTTCTGGG CGGGGGGCGT
3301 GGGGAGCTGG GGACCCGGAG CGCACTGGGG ACTGGGGAGG GGCCGCAGCT
3351 TGGGCCGGAG GGAAGAGGGG ACTTGAAGAA GGGGAGCCCC GCGCGCGCGG
3401 CTGTGGGCTT GGGGACCGGG GACTTCTCGC GCCATCCCCA GGAACGCCAG
3451 GCAAGGTCTG GGGAACAAAA GAGGAAGCTG CCCCCAGAGA GCCGGAGCTC
3501 GACTGNACTC CC 3'

Figure 4

5'

1 CTTGGTGCCG CATGCATCGT GGTGCTCATC TTTCTGGCCT TCCAGCAGAG
51 GGCATATGTG GCCCCTGCCA ACCTGCCTGC TCTCCTGCTG TTGCTACTAC
101 TGTATGGCTG GTCGATCACA CCGCTCATGT ACCCAGCCTC CTTCTTCTTC
151 TCCGTGCCCA GCACAGCCTA TGTGGTGCTC ACCTGCATAA ACCTCTTTAT
201 TGGCATCAAT GGAAGCATGG CCACCTTTGT GCTTGAGCTC TTCTCTGATC
251 AGAAGCTGCA GGAGGTGAGC CGGATCTTGA AACAGGTCTT CCTTATCTTC
301 CCCACTTCTG CTTGGGCCGG GGGCTTATTG ACATGGTGCG GNAACCAGGC
351 CATGGCTGAT GCCTTTGANC CCTTGGGAAA AAGGCAGTTC AAGTACCCTG

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 401  NCTTGGAAGG TGGCGGAAGA ACCTTTTGGC ATGGGAACAG GGCCCCTTTT
 451  CCTTCTCTTC ACACTANTGT TCAAGCACCG AAGCCAACTC NTGCCACAAG
 501  CCCAGGTAAG GTCTCTGCCA CTCCTGGAGA GAGACGAGGA TGTAGCCCGT
 551  GAACGGGAGC GGGTGGTCCA AGGAGCCACC CAGGGGGATG TGTTGGTGCT
 601  GAGGAACTTG ACCAAGGTAT ACCGTGGGCA GAGGATGCCA GCTGTTGACC
 651  GCTTGTGCCT GGGGATTCCC CCTGGTGAGT GTTTTGGGCT GCTGGGTGTG
 701  AACGGAGCAG GGAAGACGTC CACGTTTCGC ATGGTGACGG GGGACACATT
 751  GGCCAGCAGG GGCGAGGCTG TGCTGGCAGG CCACAGCGGG CCCGGGAACC
 801  CAGTGTGCGC ACCTCNAGGG CAGGCNCAGC GTGGCCCGGG AACCCAGTGC
 851  TGCGCACCTA AGCATGGGAT ACTGCCCTNA ATCCGATGCC ATCTTTGAGC
 901  TGCTGACGGG CCGCGAGCAC CTGGAGCTGC TTGCGCGCCT GCGCGGTGTC
 951  CCGGAGGCCC AGGTTGCCCA NACCGNTGGC TCGGGCCTGG CGCGTCTGGG
1001  ACTCTCATGG TACGCAGACC GGCCTGCAGG CACCTACAGG AACCTGCCCG
1051  GGCGGCCGCT CGAGCCCNTA NNTGAAGTA  3'
```

Figure 4b

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...CTCCTGCCAC AGTTAGTGAG GTCTATGGAG AGGGTGGCAG GGGCCAAGGA
     CCTACTTTAA GCCCACAGAT ATTCTGTCCC CAGGCCCAGG GTGAGGTCTC...
```

Figure 5

CDNA-sequences of lipid sensitive Genes:

ABCB9, ABCA6, ABCC4, ABCA1, ABCD2, ABCB1, ABCB4, ABCC2, ABCD1, ABCC1, ABCB6, ABCB11, ABCG2, ABCC5, ABCA5, ABCG1, ABCA3

ABCB9 GENBANK:U66676

GCCAATGNCACGGTTTCATCATGGAACTCCAGGACGGCTACAGCACAGAGACAGGGGAGA
AGGGCGCCCAGCTGTCAGGTGGCCAGAAGCAGCGGGTGGCCATGGCCGNGGCTCTGGTGC
GGAACCCCCCAGTCCTCATCCTGGATGAAGCCACCAGCGCTTTGGATGCCGAGAGCGAGT
ATCTGATCCAGCAGGCCATCCATGGCAACCTGTCAGAAGCACACGGTACTCATCATCGCG
CACCGGCTGAGCACCGTGGAGCACGCGCACCTCATTGTGGTGCTGGACAAGGGCCGCGTA
GTGCAGCAGGGCACCCACCAGCAGCTTGCTTGCCCCAGGGCGGGCTTTTACGGCAAGCTN
GTTGCAGCGGCAGATGTGGGGTTTCAAGGCCGCAGACTTCACAGCTGGCCACAACGAGCC
TGTAGCCAACGGGTCACAAGGCCTGATGGGGGGCCCCTCCTTCGCCCGGTGGCAGAGGAC
CCGGTGCCTGCCTGGCAGATGTGCCCACGGAGGTTTCCAGCTGCCCTACCGAGCCCAGGC
CTGCAGCACTGAAAGACGACCTGCCATGTCCCATGATCACCGCTTNTGCAATCTTGCCCC
TGGTCCCTGCCCCATTCCCAGGGCACTCTTACCCCNNNCTGGGGGATGTCCAAGAGCATA
GTCCTCTCCCCATACCCCTCCAGAGAAGGGGCTTCCCTGTCCGGAGGGAGACACGGGGAA
CGGGATTTTCCGTCTCTCCCTCTTGCCAGCTCTGTGAGTCTGGCCAGGGCGGGTAGGGAG
CGTGGAGGGCATCTGTCTGCCAATTGCCCGCTGCCAATCTAAGCCAGTCTCACTGTGACC
ACACGAAACCTCAACTGGGGGAGTGAGGAGCTGGCCAGGTCTGGAGGGGCCTCAGGTGCC
CCCAGCCCGGCACCCAGCTTTCGCCCCTCGTCAATCAACCCCTGGCTGGCAGCCGCCCTC
CCCACACCCGCCCCTGTGCTCTGCTGTCTGGAGGCCACGTGGACCTTCATGAGATGCATT
CTCTTCTGTCTTTGGTGGANGGGATGGTGCAAAGCCCAGGATCTGGCTTTGCCAGAGGTT
GCAACATGTTGAGAGAACCCGGTCAATAAAGTGTACTACCTCTTACCCCT

ABCA6 GENBANK:U66680

TCTTAGATGAGAAACCTGTTATAATTGCCAGCTGTCTACACAAAGAATATGCAGGCCAGA
AGAAAAGTTGCTTTTCAAAGAGGAAGAAGAAAATAGCAGCAAGAAATATCTCTTTCTGTG
TTCAAGAAGGTGAAATTTTGGGATTGCTAGGACCCAATGGTGCTGGAAAAAGTTCATCTA
TTAGAATGATATCTGGGATCACAAAGCCAACTGCTGGAGAGGTGGAACTGAAAGGCTGCA
GTTCAGTTTTGGGCCACCTGGGGTACTGCCCTCAAGAGAACGTGCTGTGGCCCATGCTGA
CGTTGAGGGAACACCTGGAGGTGTATGCTGCCGTCAAGGGGCTCAGGAAAGCGGACGCGA
GGCTCGCCATCGCAAGATTAGTGAGTGCTTTCAAACTGCATGAGCAGCTGAATGTTCCTG
TGCAGAAATTAACAGCAGGAATCACGAGAAAGTTGTGTTTTGTGCTGAGCCTCCTGGGAA
ACTCACCTGTCTTGCTCCTGGATGAACCATCTACGGGCATAACCCCACAGGGCAGCAGCA
AATGTTGGCAGGCAATCCAGGCAGTCGTTAAAAACACAGAGAGAGGTGTCCTCCTGACCA
CCCATAACCTGGCTGAGGCGGAAGCCTTGTGTGACCGTGTGGCCATCATGGTGTCTGGAA
GGCTTAGATGCATTGGCTCCATCCAACACCTGAAAAACAAACTTGGCAAGGATTACATTC
TAGAGCTAAAAGTGAAGGAAACGTCTCAAGTGACTTTGGTCCACACTGAGATTCTGAAGC

TTTTCCCACAGGCTGCAGGGCAGGAAAGGTATTCCTCTTTGTTAACCTATAAGCTGCCCC
GTGGCAGACGTTTACCCTCTATCACAGACCTTTCACAAATTAGAAGCAGTGAAAGCATAA
CTTTAACCTGGAAGAATACAGCCTTTCTCCAGTGCACACTGGANAAGGTNTCCTTANAAC
CTTCCTAAANAACAGGAAGTTAGGAAATTTTGAATGAAAANNACCNCCCCCCCTCATTC
AGGTGGAACCTTAAAACCTCAAACCTAGTAATTTTTTGTTGATCTCCTATAAAACTTATG
TTTTATGTAATAATTAATAGTATGTTTAATTTTAAAGATCATTTAAAATTAACATCAGGT
ATATTTTGTAAATTTAGTTAACAAATACATAAATTTTAAAATTATTCTTCCTCTCAAACA
TAGGGGTGATAGCAAACCTGTGATAAAGGCAATACAAAATATTAGTAAAGTCACCCAAAG
AGTCAGGCACTGGGTATTGTGGAAATAAAACTATATAAACTTAA

ABCC4 GENBANK:U66682

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TAGTTGTATTCCTTGTATGAAAAAGCGTCCCTGGTACTACAATAAGTCTTTCGTGAAAGG
AGTGTAATCCTAACAACAACTCAGGAAAGTATTTGAAAAGAATACTGGATAAGGAAAAA
CCTGCAGCTACTCCTGCTATTTCAAGACATTGCCTACAAGTGGTTGGTGTGGTCTCTGTG
GCTGTGGCCGTGATTCCTTGGATCGCAATACCCTTGGTTCCCCTTGGAATCATTTTCATT
TTTCTTCGGCGATATTTTTTGGAAACGTCAAGAGATGTGAAGCGCCTGGAATCTACAAGT
GAGTATGGAAACTCGGGTTGGTATAGACATGCTAGCTAGTTTCCATTTATGCCATAAATT
ACAGAGACCCCCTGAAATTCGGCAGACTCTGTCTTCCAGAATTTCTCTAACATTAGGTAA
TTGAACGTATTGGCCATTATGAATCATTGTGTCCCTTAGAGCATGTGGAATTGATAGCCT
GCAACGTGTAACTTTGCATTTGGAATAAGGAAGGAGTGAAGGCCATATGGGGAGTAATAT
TCTACAGGAATGTCAGCACTGTGAAGACAGGGACTC

ABCA1 Acc.Nr.: AJ012376 GENBANK:HSA012376

CAAACATGTCAGCTGTTACTGGAAGTGGCCTGGCCTCTATTTATCTTCCTGATCCTGATC
TCTGTTCGGCTGAGCTACCCACCCTATGAACAACATGAATGCCATTTTCCAAATAAAGCC
ATGCCCTCTGCAGGAACACTTCCTTGGGTTCAGGGGATTATCTGTAATGCCAACAACCCC
TGTTTCCGTTACCCGACTCCTGGGGAGGCTCCCGGAGTTGTTGGAAACTTTAACAAATCC
ATTGTGGCTCGCCTGTTCTCAGATGCTCGGAGGCTTCTTTTATACAGCCAGAAAGACACC
AGCATGAAGGACATGCGCAAAGTTCTGAGAACATTACAGCAGATCAAGAAATCCAGCTCA
AACTTGAAGCTTCAAGATTTCCTGGTGGACAATGAAACCTTCTCTGGGTTCCTGTATCAC
AACCTCTCTCTCCCAAAGTCTACTGTGGACAAGATGCTGAGGGCTGATGTCATTCTCCAC
AAGGTATTTTTGCAAGGCTACCAGTTACATTTGACAAGTCTGTGCAATGGATCAAAATCA
GAAGAGATGATTCAACTTGGTGACCAAGAAGTTTCTGAGCTTTGTGGCCTACCAAGGGAG
AAACTGGCTGCAGCAGAGCGAGTACTTCGTTCCAACATGGACATCCTGAAGCCAATCCTG
AGAACACTAAACTCTACATCTCCCTTCCCGAGCAAGGAGCTGGCCGAAGCCACAAAAACA
TTGCTGCATAGTCTTGGGACTCTGGCCCAGGAGCTGTTCAGCATGAGAAGCTGGAGTGAC
ATGCGACAGGAGGTGATGTTTCTGACCAATGTGAACAGCTCCAGCTCCTCCACCCAAATC
TACCAGGCTGTGTCTCGTATTGTCTGCGGGCATCCCGAGGAGGGGGGCTGAAGATCAAG
TCTCTCAACTGGTATGAGGACAACAACTACAAAGCCCTCTTTGGAGGCAATGGCACTGAG

GAAGATGCTGAAACCTTCTATGACAACTCTACAACTCCTTACTGCAATGATTTGATGAAG
AATTTGGAGTCTAGTCCTCTTTCCCGCATTATCTGGAAAGCTCTGAAGCCGCTGCTCGTT
GGGAAGATCCTGTATACACCTGACACTCCAGCCACAAGGCAGGTCATGGCTGAGGTGAAC
AAGACCTTCCAGGAACTGGCTGTGTTCCATGATCTGGAAGGCATGTGGGAGGAACTCAGC
CCCAAGATCTGGACCTTCATGGAGAACAGCCAAGAAATGGACCTTGTCCGGATGCTGTTG
GACAGCAGGGACAATGACCACTTTTGGGAACAGCAGTTGGATGGCTTAGATTGGACAGCC
CAAGACATCGTGGCGTTTTTGGCCAAGCACCCAGAGGATGTCCAGTCCAGTAATGGTTCT
GTGTACACCTGGAGAGAAGCTTTCAACGAGACTAACCAGGCAATCCGGACCATATCTCGC
TTCATGGAGTGTGTCAACCTGAACAAGCTAGAACCCATAGCAACAGAAGTCTGGCTCATC
AACAAGTCCATGGAGCTGCTGGATGAGAGGAAGTTCTGGGCTGGTATTGTGTTCACTGGA
ATTACTCCAGGCAGCATTGAGCTGCCCCATCATGTCAAGTACAAGATCCGAATGGACATT
GACAATGTGGAGAGGACAAATAAAATCAAGGATGGGTACTGGGACCCTGGTCCTCGAGCT
GACCCCTTTGAGGACATGCGGTACGTCTGGGGGGGCTTCGCCTACTTGCAGGATGTGGTG
GAGCAGGCAATCATCAGGGTGCTGACGGGCACCGAGAAGAAAACTGGTGTCTATATGCAA
CAGATGCCCTATCCCTGTTACGTTGATGACATCTTTCTGCGGGTGATGAGCCGGTCAATG
CCCCTCTTCATGACGCTGGCCTGGATTTACTCAGTGGCTGTGATCATCAAGGGCATCGTG
TATGAGAAGGAGGCACGGCTGAAAGAGACCATGCGGATCATGGGCCTGGACAACAGCATC
CTCTGGTTTAGCTGGTTCATTAGTAGCCTCATTCCTCTTCTTGTGAGCGCTGGCCTGCTA
GTGGTCATCCTGAAGTTAGGAAACCTGCTGCCCTACAGTGATCCCAGCGTGGTGTTTGTC
TTCCTGTCCGTGTTTGCTGTGGTGACAATCCTGCAGTGCTTCCTGATTAGCACACTCTTC
TCCAGAGCCAACCTGGCAGCAGCCTGTGGGGGCATCATCTACTTCACGCTGTACCTGCCC
TACGTCCTGTGTGTGGCATGGCAGGACTACGTGGGCTTCACACTCAAGATCTTCGCTAGC
CTGCTGTCTCCTGTGGCTTTTGGGTTTGGCTGTGAGTACTTTGCCCTTTTTGAGGAGCAG
GGCATTGGAGTGCAGTGGGACAACCTGTTTGAGAGTCCTGTGGAGGAAGATGGCTTCAAT
CTCACCACTTCGGTCTCCATGATGCTGTTTGACACCTTCCTCTATGGGGTGATGACCTGG
TACATTGAGGCTGTCTTTCCAGGCCAGTACGGAATTCCCAGGCCCTGGTATTTTCCTTGC
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Fragment 698739

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Fragment 1133530

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Fragment 1125168

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Fragment 168043

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Huwhite2

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1780 1785 1790

Leu Phe Ala Met Ala Val Glu Gly Val Val Phe Phe Leu Ile Thr Val
1795 1800 1805

Leu Ile Gln Tyr Arg Phe Phe Ile Arg Pro Arg Pro Val Asn Ala Lys
1810 1815 1820

Leu Ser Pro Leu Asn Asp Glu Asp Glu Asp Val Arg Arg Glu Arg Gln
1825 1830 1835 1840

Arg Ile Leu Asp Gly Gly Gly Gln Asn Asp Ile Leu Glu Ile Lys Glu
1845 1850 1855

Leu Thr Lys Ile Tyr Arg Arg Lys Arg Lys Pro Ala Val Asp Arg Ile
1860 1865 1870

Cys Val Gly Ile Pro Pro Gly Glu Cys Phe Gly Leu Leu Gly Val Asn
1875 1880 1885

Gly Ala Gly Lys Ser Ser Thr Phe Lys Met Leu Thr Gly Asp Thr Thr
1890 1895 1900

Val Thr Arg Gly Asp Ala Phe Leu Asn Arg Asn Ser Ile Leu Ser Asn
1905 1910 1915 1920

Ile His Glu Val His Gln Asn Met Gly Tyr Cys Pro Gln Phe Asp Ala
1925 1930 1935

Ile Thr Glu Leu Leu Thr Gly Arg Glu His Val Glu Phe Phe Ala Leu
1940 1945 1950

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Ile Arg Lys Leu Gly Leu Val Lys Tyr Gly Glu Lys Tyr Ala Gly Asn
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Tyr Ser Gly Gly Asn Lys Arg Lys Leu Ser Thr Ala Met Ala Leu Ile
1985 1990 1995 2000

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2005 2010 2015

Pro Lys Ala Arg Arg Phe Leu Trp Asn Cys Ala Leu Ser Val Val Lys
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Glu Gly Arg Ser Val Val Leu Thr Ser His Ser Met Glu Glu Cys Glu
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Leu
65

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<213> Human

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<213> Human

<220>
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<210> 18
<211> 235
<212> DNA
<213> Human

<220>
<223> human cDNA

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<211> 636
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<213> Human

<220>
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<210> 20
<211> 2911
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<213> Human

<220>
<223> human cDNA of ABCA8 (ABC-new)

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<211> 100
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<213> Human

<220>
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<212> DNA
<213> Human

<400> 22
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<210> 23
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<212> DNA
<213> Human

<220>
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